Vol. 40 No. 7

BULLETIN

OF THE

TORREY BOTANICAL CLUB

JULY, 1913

Studies on the West Indian Vernonieae, with one new species from Mexico

HENRY ALLAN GLEASON

Seven years ago, in the Revision of the North American Vernonieae,* forty-three species and one variety of *Vernonia* were recognized from the West Indies, together with several species of other genera. Since that time, the New York Botanical Garden has been actively engaged in the systematic exploration of these islands, especially Cuba, and has accumulated much valuable material. Director N. L. Britton has recently extended to me the facilities of the Garden, and has given me the opportunity to examine the new material in this tribe of Carduaceae. The results of this study are herewith presented.

Of the forty-four species and varieties recognized in the revision, three have been found untenable and are reduced to synonymy: *V. sublanata* Gleason, *V. Thomae* Benth., and *V. Sintenisii* (Urban) Gleason. The variety has been raised to specific rank. Four old species, not recognized in the revision, are shown by the recent collections to be distinct, and are admitted as species: *V. acuminata* Lessing, *V. Ottonis* Sch.-Bip., *V. Wrightii* Sch.-Bip., and *V. fruticosa* (L.) Sw. For the identification of the latter, we are indebted to Dr. Urban, who has also described a new species, *V. Tuerckheimii*. The collections at the Garden, supplemented by some material loaned by the Gray Herbarium and the Missouri Botanical Garden, are also found to contain seventeen

* Bull. N. Y. Bot. Gard. 4: 144–243. 1906.

[The BULLETIN for May 1913 (40: 193–248. *pls. 9–14*) was issued May 20; the BULLETIN for June 1913 (40: 249–304. *pls. 15–19*) was issued June 18.]

new species, which are here described. The total number of species of *Vernonia* recognized from the West Indies at the present time is therefore sixty-three. In addition, an Asiatic species escaped or adventive in Guadeloupe has been found to differ from *Vernonia* in particulars important enough to warrant its segregation, and a new species of the related genus *Eremosis* has been described from Mexico.

CYANTHILLIUM Blume, Bijdr. Flora Nederl. Indie 889. 1826

Isonema Cass. Bull. Soc. Philom. **1817**: 152. 1817. Not *Isonema* R. Br., 1809.

Cyanthyllium Blume, Flora Javae 1: vi. 1828.

Cyanopis Blume, Flora Javae 1: vi. 1828; DC. Prodr. 5: 69. 1836.

Cyanopsis Endl. Ench. 232. 1841. Not *Cyanopsis* Cass. Bull. Soc. Philom. **1817**: 200. 1817.

Claotrachelus Zoll. Nat. en Geneesk. Arch. Neêrl. Indie **2**: 565. 1845, *fide* Hoffman, in Engl. & Prantl, Nat. Pfl.-Fam.

The genus differs from *Vernonia* in its very fragile, uniseriate pappus, and its 4–5-angled achenes.

Cyanthillium chinense (Lam.) comb. nov.

Eupatoria conyzoides foliis glabris summo caule ramosior, lamii folio flore purpureo Pluk. Phytographia *pl. 177. f. 2.* 1691.

Conyza chinensis Lam. Encyc. 2: 83. 1786.

Conyza patula Ait. Hort. Kew. 3: 184. 1789.

Isonema ovata Cass. Dict. Sc. Nat. 24: 25. 1822.

Conyza odorata Willd. in Spreng. Syst. 3: 511. 1826.

Cyanthillium villosum Blume, Bijdr. Flora Nederl. Indie 889. 1826.

Cyanthillium pubescens Blume, Bijdr. Flora Nederl. Indie 890. 1826.

Centratherum chinense Less. Linnaea **4**: 320. 1829.

Vernonia chinensis Less. Linnaea **6**: 105, 674. 1831.

Cyanopis pubescens DC. Prodr. 5: 69. 1836.

Cyanopis villosa DC. Prodr. 5: 69. 1836.

Claotrachelus rupestris Zoll. Nat. en Geneesk. Arch. Neêrl. Indie **2**: 565. 1845.

As long ago as 1829 Lessing mentioned a specimen of this species from the "insulis Caribaeis" in Willdenow's herbarium. The herbarium of the New York Botanical Garden now contains a specimen from Guadeloupe, *Duss 4032*, where it occurs in a single locality. It is therefore admitted as a member of the North American Flora.

SPECIES-GROUP ARBORESCENTES

Vernonia icosantha DC. Occurs also in Guadeloupe Island, *Duss 2812*.

Vernonia arborescens (L.) Sw. This species, typically of Jamaica, occurs also in Grand Cayman, *Hitchcock*, in the herbarium of the Missouri Botanical Garden.

Vernonia amaranthina sp. nov.

Apparently herbaceous and freely branched, height not reported; stem and branches straight, striate, closely and softly pubescent or tomentulose, the branches ascending; leaves spreading, firm, rather dull green, ovate-lanceolate to narrowly oblong-elliptical, the principal ones 7–9 cm. long by 2–3 cm. wide, acute or short-acuminate, entire or somewhat irregular at the margin, but not serrate, narrowed to an acute base, minutely papillose-pubescent and scabrellate to the touch above, pubescent, especially along the veins, and minutely glandular beneath, veins conspicuous, elevated beneath, the lateral ones strongly ascending; petioles definite, 5–10 mm. long, pubescent or tomentulose; inflorescence freely branched and spreading; cymes short, naked below and bearing 2–5 heads aggregated near the tip, or separated by internodes less than 1 cm. long; rameal leaves resembling the cauline but smaller, those subtending the cymes narrowly oblanceolate, 1–2 cm. long, those subtending the heads linear to narrowly oblanceolate, shorter than or barely equaling the involucre; heads 21–29-flowered, sessile; corolla purple; involucre campanulate, about 5 mm. high, its scales purplish, thinly pubescent, irregularly imbricated, the outer triangular to ovate, sharply acute and somewhat cuspidate, the inner oblong, rounded to a mucronulate tip; achenes densely hirsute, 1.5 mm. long; pappus white, 4 mm. long, the outer series conspicuous.

Type, *E. G. Britton 2897*, from "Greenland," Jamaica, March 13, 1908, in the Herbarium of the New York Botanical Garden. *Britton & Hollick 2133*, from Green Island, Jamaica, is also

referred here. The latter is a virgate plant 2 meters high, and the inflorescence is consequently small and composed of relatively few heads. It differs in no essential way from the type.

V. amaranthina is distinguished from *V. arborescens*, its nearest relative, by the rounded and mucronulate inner scales of the involucre and its short contracted cymes, contrasting with the acute or acuminate inner scales and elongated cymes of *V. arborescens*.

SPECIES-GROUP ARARIPENSES

A. Leaves of a linear type, 1–5 mm. wide, one-nerved, more or less revolute.
1. Leaves linear to narrowly lanceolate, 3–5 mm. wide; inner scales broadest above the base, purplish or brown; middle and outer scales glabrate; pappus tawny or pale brown. *Vernonia araripensis.*
2. Leaves 1–4 mm. wide, linear; inner scales broadest near the base; all scales densely strigose, pale-green; pappus white or very pale yellowish-brown.
   a. Leaves 3–8 cm. long, densely papillose-pubescent above; inflorescence divaricately branched; heads 18–21-flowered. *Vernonia stenophylla.*
   b. Leaves 1–3 cm. long, strigose-hispid above; inflorescence slender, virgate; heads 11-flowered. *Vernonia corallophila.*

B. Leaves 4–20 mm. wide, narrowly oblong to oblong-obovate, mucronate; inner scales broadest above the base; all scales strigose-pubescent; pappus white or nearly so.
1. Leaves narrowly oblong with parallel sides, without conspicuous lateral veins beneath. *Vernonia angustata.*
2. Leaves broadest near or above the middle, veiny. *Vernonia gnaphaliifolia.*

The status of the first three species in the key above is still open to question and may require future adjustment. Gardner's type locality for *V. araripensis* was in Brazil, and it is scarcely probable that the same species occurs in the Antilles, especially as far as Cuba or Santo Domingo. Authentic specimens of Lessing's *V. stenophylla* have not been seen, and the plants referred to his name differ in some slight features from the original description. Both species are accepted solely on the authority of certain European students of the genus.

Vernonia corallophila sp. nov.

Stem erect, herbaceous, 4 dm. high, virgate, finely striate, strigose-pubescent; leaves sessile, narrowly linear, rigid, revolute, one-nerved, 1–3 cm. long, strigose-hispid and punctate above, densely strigose-pubescent beneath; inflorescence cylindrical or sparingly branched; heads 11-flowered, in the axils of the upper leaves or rarely 2 or 3 together on a short ascending lateral cyme; bracteal leaves resembling the cauline, but reduced in size and the upper barely exceeding the heads; involucre turbinate, 5–6 mm. high, its scales all straight, erect, very loosely imbricated, strigose-pubescent, the outer subulate, the inner narrowly oblong, long-acuminate; achenes pubescent, 2 mm. long; pappus nearly white, the inner series 4–5 mm. long.

Type, *Britton 1939*, from a coral limestone beach at the United States Naval Station, Guantanamo Bay, Oriente, Cuba, March 17–30, 1909, deposited in the Herbarium of the New York Botanical Garden.

The species is readily distinguished by its slender virgate inflorescence and small leaves from *V. stenophylla* with its long leaves and divaricate inflorescence.

Vernonia angustata sp. nov.

Vernonia sublanata angustata Gleason, Bull. N. Y. Bot. Gard. 4: 177. 1906.

Herbaceous, height not stated; stems slender, puberulent or thinly pubescent, sparingly branched above; leaves thin, flat, spreading, narrowly oblong with parallel sides, 3–5 cm. long, 4–8 mm. wide, obtuse or rounded, mucronate, entire, acute at the base, thinly pubescent above, closely gray-tomentose beneath, one-nerved; petioles 1–2 mm. long; cymes few, widely spreading or horizontal, simple or sparingly branched, bearing 2–7 heads each; internodes 1–2 cm. long, depressed or gently curved between the heads; bracteal leaves like the cauline, but gradually reduced in size, and the upper only 1 cm. long; heads sessile, distinctly secund, about 18-flowered; involucre broadly campanulate, 6–7 mm. high, its scales loosely and irregularly imbricated, strigose-pubescent, acuminate or sharply acute, the outer lanceolate, the inner narrowly oblong; achenes pubescent, 2 mm. long; pappus white, the inner series 6 mm. long.

VERNONIA GNAPHALIIFOLIA Rich. in Sagra, Hist. Fis. Pol. Nat. Cuba **11**: 34. 1850

Vernonia sublanata Gleason, Bull. N. Y. Bot. Gard. **4**: 177. 1906.

Dr. Urban has established the identity of these two species by the examination of authentic material, and they are accordingly united here. The species appears to be common and widely distributed in Cuba from the province of Havana eastward.

SPECIES-GROUP DIVARICATAE

The history of this group of Vernonieae begins with the publication of *V. divaricata* by Swartz in 1806. This was followed in 1831 by Lessing's *V. acuminata*, and no further addition was made to the group until 1906, when Gleason described *V. albicoma* and *V. expansa*. During the last half-century *V. divaricata* and *V. acuminata* have been usually considered identical. The whole group, so far as known, is confined to Jamaica, and the ample collection in the Herbarium of the New York Botanical Garden includes seven distinct species.

At the present time the chief difficulty lies in determining to which of these seven species the old names *divaricata* and *acuminata* belong. Certain characters given by the authors in their original descriptions serve to exclude one or another of the species, and by this process of exclusion it is possible to arrive finally at a reasonable conclusion. It may be safely affirmed that the two species described below are the only ones examined to which these names can consistently be given, without doing violence to some important feature of the original description.

VERNONIA DIVARICATA Sw. Fl. Ind. Occ. 3: 1319. 1806

A straggling shrub, 1–2 m. high; leaves spreading, thin, bright green, elliptical or elliptical-lanceolate, 4–7 cm. long, 1.5–2.5 cm. wide, acute or subacuminate, entire, narrowed to an acute base, minutely puberulent above, thinly pubescent and finely punctate with minute pellucid glands beneath, sessile or with petioles 1–5 mm. long; inflorescence of numerous, lax, loosely flowered, divaricate cymes, bearing each 5–15 secund heads, and frequently prolonged into a leafy shoot; bracteal leaves oblanceolate to narrowly oblong, barely exceeding the 11–13-flowered heads; involucre campanulate, about 4 mm. high; scales thinly strigose-

pubescent, the inner oblong, gradually narrowed to a rounded tip, the outer triangular-ovate, obtuse or acute.

The following specimens are referred here: JAMAICA: *Harris 8205*, altitude 500 m.; *Britton & Hollick 1996*, altitude 700 m.; *Britton 431, 679; Cockerell.*

VERNONIA ALBICOMA Gleason, Bull. N. Y. Bot. Gard. **4**: 185. 1906

Stem woody, slender; leaves thin, bright green, elliptical or elliptical-lanceolate, 5–6 cm. long, 1.5–2.3 cm. wide, acuminate, subentire, tapering at the base, glabrate above, very finely pubescent or glabrous beneath; petioles 5–7 mm. long; upper leaves somewhat reduced; inflorescence of 1–3 curved spreading terminal cymes bearing each 5–10 sessile secund 18-flowered heads; bracteal leaves lance-oblong, the middle ones about equaling the involucre, which is broadly campanulate or hemispheric, 5 mm. high; scales erect, loosely and irregularly imbricated, nearly glabrous, the outer lanceolate, acuminate, the inner oblong, acute; pappus white.

Campbell 6152, from the foot of Long Mountain, Jamaica, altitude 115 m.

VERNONIA ACUMINATA Less. Linnaea **6**: 663. 1831

Shrubby, height not stated; stem finely cinereous-puberulent; leaves thin, bright green, broadly ovate-lanceolate, 6–9 cm. long and half as wide, long-acuminate, entire, acute at the base, very sparsely and minutely puberulent on both sides, on petioles about 5 mm. long; inflorescence of numerous widely spreading freely branched cymes, the ultimate branches bearing 3–7 heads; rameal leaves lanceolate, 2–3 cm. long; bracts oblong-lanceolate to linear, those at the ends of the cymes only 6 mm. long; heads 18-flowered; involucre campanulate, about 5 mm. high, its scales all obtuse or the outer acute, pubescent, especially the outer series, the exposed portion of the inner series oblong.

Wight 20, from wooded hillsides, Port Antonio, Jamaica.

VERNONIA EXPANSA Gleason, Bull. N. Y. Bot. Gard. **4**: 186. 1906

A straggling shrub 2.5–3 m. high, branching above; leaves numerous, rather crowded, thin, divaricate, ovate, 3–5 cm. long, 1.7–2.7 cm. wide, acute or subacuminate, entire, obtuse or broadly rounded at base, sparsely and finely pubescent, or nearly glabrous above; petioles 3 mm. long; upper and rameal leaves smaller, broadly elliptical, obtuse; inflorescence of several short cymes bearing each 3–6 heads; bracteal leaves equaling or much shorter

than the involucre; heads crowded, sessile, 11-flowered; involucre broadly campanulate, 4 mm. high; scales all appressed, closely imbricated, glabrous or nearly so, rounded at the tip, the outer broadly ovate, the exposed portion of the inner ovate.

JAMAICA: *Harris 8796*, near Troy, altitude 2,000 ft., *Britton & Hollick 1956*, woods, Bluefields Mountain, altitude 750 m.

Vernonia pluvialis sp. nov.

Apparently erect and branched only near the top, height 4 dm. or more; stem finely striate, glabrate, or thinly pubescent, especially above; leaves spreading, or usually ascending, firm and rigid, oblong-ovate to subrhomboid, broadest conspicuously above the middle, the principal ones 3–5 cm. long by 1.1–1.9 cm. wide, acute, entire and subrevolute, gradually narrowed to an obtuse base, minutely puberulent on both sides and glandular-punctate below; veins not prominent, sessile or on petioles 1–2 mm. long; inflorescence of several short few-headed cymes in the axils of the upper leaves, forming a compact leafy panicle; bracteal leaves barely exceeding the heads, ovate to ovate-lanceolate; heads crowded in clusters of 2–7, or sometimes single, sessile, not at all secund, 8- (rarely 5-) flowered; involucre narrowly campanulate or subcylindric, 5–7 mm. high, its scales puberulent, well imbricated in several ranks, the outer ovate-triangular, acute or apiculate, the inner becoming oblong and slightly narrowed to an obtuse or rounded tip; achenes densely hirsute, not mature in the specimens examined; inner pappus pale brown, 4–5 mm. long on the immature achenes.

Type collected by Forrest Shreve on Blue Mountain Peak, Jamaica, May 14, 1906, and deposited in the Herbarium of the New York Botanical Garden. Other sheets in the same herbarium are *Nichols 20*, from moist woods, Morce's Gap, altitude 5,000 feet, *Nichols 120*, from the summit of Blue Mountain Peak, and *E. G. Britton 3856*, from Sir John Peak.

Vernonia proclivis sp. nov.

Shrubby, about 2 m. high; stem sparingly branched, finely striate, glabrate or thinly puberulent; cauline leave sconspicuously reflexed, bright green above, pale green beneath, thin, elliptic-oblong, widest at or near the middle, 6–8 cm. long by 2–3 cm. wide, distinctly acuminate, entire, gradually narrowed or subacuminate at base, glabrous and finely glandular-punctate with transparent globules above and minutely black-punctate beneath; veins promi-

nent beneath; petioles 3–4 mm. long; rameal and bracteal leaves similar, but much smaller; inflorescence cylindrical or pyramidal, composed of numerous short, divaricate, irregularly branched cymes, forming a terminal panicle, and bearing each 4–10 sessile, crowded, 8-flowered heads near their tips; involucre narrowly campanulate, 4–5 mm. high, its scales erect, regularly imbricated in several ranks, thinly puberulent or glabrate, the outer ovate, with acute or apiculate tips, the inner oblong, slightly narrowed to a rounded tip; flowers purple to pale lavender; immature achenes densely pubescent; outer pappus pale brown, 0.4 mm. long, the inner brown, 4 mm. long.

Type, *Britton 102*, from Morce's Gap, near Cinchona, Jamaica, September 2–10, 1906, in the Herbarium of the New York Botanical Garden. Three other sheets in the same herbarium agree with it perfectly and are referred to the same species: *Marble 188*, from Cinchona, *E. G. Britton 3856*, from the Blue Mountains, and *Britton 4055*, from the Parish of St. Thomas. The latter was collected in March; the achenes are all discharged and the dry and brown involucre is widely open.

Vernonia reducta sp. nov.

Shrubby, freely branched above, height not stated; stem striate, glabrate below, becoming puberulent in the inflorescence; leaves spreading, firm, bright green above, paler beneath, narrowly elliptical-oblong or somewhat oblanceolate, broadest at or near the middle, the principal ones 4–4.5 cm. long by 1.2–1.6 cm. wide, sharply acute or subacuminate, entire or finely denticulate, gradually narrowed at the base, minutely puberulent on both sides and finely glandular-punctate beneath; petioles 2–5 mm. long; cymes numerous, terminating the stem and the upper branches, and forming a large loose pyramidal panicle; rameal and bracteal leaves resembling the cauline, but becoming narrower and smaller toward the ends of the branches, and finally barely exceeding the heads; heads crowded in clusters of 2–7, sessile or nearly so, not at all secund, 5-flowered; involucre narrowly campanulate or subcylindric, 5–6 mm. high, its scales puberulent on the back and regularly imbricated in several ranks, outer scales triangular, acute, and somewhat arachnoid-ciliate, the inner becoming oblong-linear, puberulent or glabrate, narrowed to an obtuse apex; achenes about 2 mm. long, hirsute; pappus rufescent, the outer series 0.5 mm., the inner 4–5 mm. long.

Type, *Britton 203*, from Sir John Peak, Jamaica, September

2–10, 1906, in the Herbarium of the New York Botanical Garden. Two other sheets in the same herbarium are *Britton 151*, from New Haven Gap, and *Shreve*, from Sir John Peak.

The seven species of the group may be distinguished by the following key:

A. Heads more or less secund, 11–18-flowered; involucre campanulate to hemispheric, distinctly spreading when press-dried; its scales loosely and irregularly imbricated in few ranks.
  1. Principal leaves about 3 times as long as broad.
    a. Heads 11–13-flowered; pappus brown. *Vernonia divaricata.*
    b. Heads 18-flowered; pappus white. *Vernonia albicoma.*
  2. Principal leaves twice as long as broad, or less.
    a. Leaves broadly ovate-lanceolate, distinctly acuminate; exposed portion of inner involucral scales oblong; heads 18-flowered; cymes freely branched. *Vernonia acuminata.*
    b. Leaves broadly ovate, acute or subacuminate; exposed portion of inner involucral scales ovate; heads 11-flowered; cymes sparingly branched. *Vernonia expansa.*

B. Heads not at all secund, 5–8-flowered; involucre narrowly campanulate or subcylindrical, even when press-dried; its scales rather uniformly imbricated in several ranks.
  1. Leaves spreading or ascending, nearly or quite sessile, distinctly oblong-obovate or subrhomboidal, 30–50 mm. long, acute; heads 8- (rarely 5-) flowered. *Vernonia pluvialis.*
  2. Leaves proportionately narrower or larger, broadest near the middle, more or less acuminate.
    a. Leaves elliptical-oblong, about 25×65 mm., conspicuously reflexed; heads 8-flowered. *Vernonia proclivis.*
    b. Leaves narrowly elliptical-oblong, about 14×45 mm., spreading; heads 5-flowered. *Vernonia reducta.*

SPECIES-GROUP FRUTICOSAE

When a sheet of Santo Domingan material came to hand, identified by Dr. Urban as authentic *V. fruticosa* Sw., it was at once seen that the species was entirely distinct from *V. rigida* and from the whole group to which *V. rigida* belongs. It was also seen that its nearest congeners were to be found among some undescribed species from eastern Cuba, with which it is accordingly grouped. As in several other species-groups, the chief similarity between the species included is in the general habit. The most

striking common feature here is the broad, rugose or bullate leaf with reticulate venation, closely invested beneath by a dense tomentum. In the Jamaican species-group *Permolles*, with similarly bullate or rugose leaves, the pubescence is densely sericeous-hirsute rather than closely tomentose, and in *V. yunquensis* the character of the involucral scales separates the species at once. The seven species may be distinguished as follows:

I. Santo Domingan species; leaves oblong-ovate, truncate or subcordate at base, obtuse, repand; bracteal leaves not reduced; cymes very flexuous. *Vernonia fruticosa.*

II. Cuban species.

A. Inner involucral scales obtuse or rounded; leaves ovate-lanceolate. *Vernonia desiliens.*

B. Inner involucral scales sharply acute or acuminate.

1. Leaves oval to elliptic-oblong, obtuse or narrowed at the base, rugose and finely papillose-pubescent above.

a. Leaves more than half as broad as long, very blunt or rounded at the apex; straggling plant with white flowers. *Vernonia calophylla.*

b. Leaves less than half as broad as long, narrowed to an obtuse or subacute apex; plant erect, with light blue flowers. *Vernonia vicina.*

2. Leaves of a lanceolate or ovate type, broadest near the truncate or subcordate base, strongly bullate and rugose above.

a. Leaves acute or barely obtuse, gray-tomentose beneath; inflorescence loose; cymes elongated; inner involucral scales broadest just below the middle. *Vernonia neglecta.*

b. Leaves obtuse or rounded; inflorescence compact; cymes abbreviated; inner involucral scales broadest near the base.

* Leaves ovate-oblong, crenate or repand, brown-tomentose beneath; involucre densely pubescent; inner scales merely acute. *Vernonia calida.*

** Leaves broadly ovate or oval, entire or somewhat revolute, closely gray-tomentose beneath; involucre thinly pubescent or glabrate; inner scales sharply acuminate. *Vernonia semitalis.*

VERNONIA FRUTICOSA (L.) Sw. Fl. Ind. Occ. 3: 1323. 1806.

Stem very slender, freely and loosely branched, glabrous or puberulent in the inflorescence; leaves firm, ovate-oblong, 1–2 cm.

long by half as wide, obtuse or rounded at the tip, irregularly crenulate or subentire, rounded or subcordate at base, dark green and thinly but softly pubescent above, densely gray-tomentose beneath; petioles 1–2 mm. long; upper and terminal branches floriferous and strongly flexuous; bracteal leaves resembling the cauline in shape and size; heads single, sessile, 21-flowered; involucre broadly turbinate or campanulate, 5 mm. high, its scales all erect or somewhat spreading, very loosely imbricated, puberulent or glabrous, the outer subulate, the inner narrowly lanceolate and long-acuminate with a subulate tip; achenes hirsute; pappus nearly white, 4 mm. long.

For the identification of this obscure species of Swartz we are indebted to Dr. Urban. The description above is based on a single sheet, *Fuertes 655*, from the Province of Barahona, Santo Domingo, at 350 m. altitude.

Vernonia desiliens sp. nov.

Herbaceous, sparingly branched, 3–5 dm. tall; stem rather prominently striate, glabrous below, becoming puberulent in and near the inflorescence; leaves firm and coriaceous, ovate-lanceolate to narrowly elliptical, the largest on the type sheet 8–8.5 cm. long and 2.3–2.9 cm. wide, obtuse, entire or obscurely and shallowly crenulate, obtuse or rounded at the base, with petioles 1–3 mm. long, rugose, glabrous and shining above; veins elevated beneath and prominently reticulated, the principal lateral ones strongly ascending and almost parallel to the leaf-margin; the interstices of the reticulations closely gray-tomentose; cymes apparently one or two, terminal and from the upper axils, spreading, 1–2 dm. long, very flexuous; bracteal leaves resembling the cauline but narrower in shape and gradually reduced in size, oblong or narrowly elliptical, 3–5 cm. long, 5–12 mm. wide, spreading, those at the extremity of the cyme oblong-linear, 2 cm. long; heads sessile, single in the axils, 1–2 cm. apart, 21-flowered; corollas purplish; involucre turbinate, thinly pubescent, 9–10 mm. high; the lower outer scales short, minute, triangular, acute and apiculate, and closely imbricated; the inner scales much longer, linear-oblong, obtuse; achenes strigose-pubescent; pappus light brown, the inner series 6 mm. long, minutely barbellate, the outer series 0.5 mm. long, somewhat paler.

Type, *Shafer 3232*, growing among rocks near water, Arroyo del Medio, Oriente, Cuba, at 450–550 m. altitude, January 20, 1910, deposited in the Herbarium of the New York Botanical Garden.

Vernonia calophylla sp. nov.

A straggling shrub, 1 m. high or less; stem slender, striate, freely and loosely branched, cinereous-puberulent below, becoming tomentose in the branches; leaves firm and rigid, ovate to subrotund, the principal ones 20–25 mm. long by 13–18 mm. wide, rounded or obtuse at the apex, entire, somewhat revolute, rounded or subcordate at base, dark green, rugose, and papillose-pubescent above, closely invested beneath with silver-gray tomentum; veins conspicuous, the lateral ones ascending and the veinlets prominently reticulated; petioles 1–2 mm. long; heads in the axils of the upper leaves, forming cymes 10–16 cm. long, sessile, 18–21-flowered; bracteal leaves resembling the cauline, but smaller, 1–1.5 cm. long and more densely pubescent above; corollas white; involucre campanulate, 5 mm. high; scales somewhat arachnoid-puberulent, especially near their tips, the outer ones narrowly triangular, subulate, the inner linear-oblong, acute; achenes pubescent, 2 mm. long; pappus nearly white, the outer series 0.8 mm., the inner 4 mm. long.

Type, *Shafer 8102*, from Camp La Gloria, south of Sierra Moa, Oriente, Cuba, December 24–30, 1910, deposited in the Herbarium of the New York Botanical Garden.

Vernonia vicina sp. nov.

An upright shrub, 3–6 dm. tall, sparingly branched; stem finely striate, glabrate below, puberulent above, and in the branches closely cinereous-tomentulose; leaves firm and rigid, spreading, dark green above, elliptic to elliptic-oblong, the principal ones 3–4.5 cm. long by 1–1.5 cm. wide, broadest at or near the middle, narrowed to an obtuse or subacute tip and to an acute base, entire, somewhat revolute, rugose above, papillose-pubescent when young, becoming glabrate with age, closely invested beneath with thin yellowish brown or cinereous tomentum, especially between the veins; lateral veins ascending, but curved and approximate near the margin; veinlets prominently reticulated; petioles curved, 1–2 mm. long, tomentose; upper leaves like the lower, but smaller, eventually only 1 cm. long by 4 mm. wide, bearing heads in the upper 4–5 axils; heads about 26-flowered; flowers light blue, involucre campanulate, 5–6 mm. high, its scales closely imbricated, glabrous or thinly puberulent, outer scales subulate, somewhat spreading, inner scales lance-oblong, sharply acute; achenes pubescent, 2 mm. long; outer pappus white, 0.8 mm. long; inner series almost white, 4 mm. long.

Type, *Shafer 8202*, from Camp La Gloria, south of Sierra Moa.

Oriente, Cuba, December 24–30, 1910, deposited in the Herbarium of the New York Botanical Garden.

Vernonia neglecta nom. nov.

V. Wrightii Griseb. Cat. Pl. Cuba 144. 1866. Not *V. Wrightii* Sch.-Bip. 1863.

Vernonia gnaphaliifolia Gleason, Bull. N. Y. Bot. Gard. 4: 178. 1906. Not *V. gnaphaliifolia* Rich. 1850.

Apparently erect and suffruticose, sparingly branched, 6–15 dm. high; stem and branches slender, obscurely striate, finely gray-tomentulose; leaves firm, flat, spreading, narrowly ovate-oblong to ovate-lanceolate, the largest on the type sheet 6–7 cm. long by 2 cm. wide, acute, or subobtuse and minutely apiculate, entire, rounded, truncate, or even subcordate at base, dark green and bullate above, papillose-puberulent when young, becoming glabrous and shining at maturity, softly gray-tomentose or almost villous beneath, especially along the elevated, prominently reticulated veins; petioles tomentose, 1–4 mm. long; cymes 2 or 3, terminal, spreading, 1.5–2.5 dm. long, straight, leafy; lower bracteal leaves ovate-lanceolate or lanceolate, about 3 cm. long by 1.3 cm. wide; the upper reduced, becoming oblong and only 1.5 cm. long; heads sessile, secund, about 21-flowered, separated by internodes 1–2 cm. long; corollas purple; involucre broadly campanulate, about 6 mm. high, its scales rather closely but irregularly imbricated, thinly pubescent or glabrate, the outer triangular and subulate, the inner ovate-lanceolate, sharply acute, somewhat scarious on the margin; achenes pubescent, 1.5 mm. long; outer pappus white, 0.9 mm. long, the inner series very pale brown or almost white, 4 mm. long.

Type, *Wright 1309*, on banks of cliffs near Monte Verde, eastern Cuba, deposited in the Gray Herbarium of Harvard University. The specimen bears two labels, one dated "Dec. 27," the other "Jan.–Jul. 1859." On the left-hand side of the same sheet is another specimen of the same species, also numbered *1309*, and collected in eastern Cuba, Sept. 1859–Jan. 1860. Another sheet of the same species in the Gray Herbarium is *Wright 2788*.

Vernonia calida sp. nov.

Shrubby, freely branched, 5 dm. tall; stem obscurely striate, thinly puberulent or finely cinereous-tomentulose in the inflorescence; leaves spreading, thick, rigid, ovate-oblong, the principal

ones 3.5–5 cm. long and 1.5–2 cm. wide, obtuse or rounded at the tip, crenate or repand, broadly rounded or subcordate at base, strongly bullate, dark green, minutely papillose-pubescent when young, soon becoming glabrate or scabrellate and shining above, densely and softly brown-tomentose beneath; petioles 1–3 mm. long; lateral veins prominent, strongly curved and soon confluent; veinlets prominently reticulated; cymes few, simple or sparingly branched, 6–12 cm. long, the rachis densely tomentose; bracteal leaves resembling the cauline in shape, but smaller, crenulate or entire, the upper 1–1.5 cm. long; heads rather crowded, single or frequently two at each node, about 21-flowered, separated by internodes 1–2 cm. long; corollas pink; involucre broadly campanulate, 7 mm. high; scales densely pubescent, closely imbricated, or somewhat spreading at the tip, the outer subulate, the inner narrowly triangular-lanceolate and acute; achenes pubescent, 1.5 mm. long; pappus yellowish brown, the outer series 0.7 mm., the inner 5 mm. in length.

Type, *Shafer 8408*, in dry soil, Sabanilla to Yamuri Arriba, Oriente, Cuba, January 30, February 1, 1911, deposited in the Herbarium of the New York Botanical Garden.

Vernonia semitalis sp. nov.

Shrubby, 6–9 dm. tall, freely branched above; stem striate, leafy, thinly brown-tomentose, especially on the younger branches; leaves numerous and crowded, thick, rigid, somewhat revolute, divaricately spreading, ovate or ovate-triangular, broadest near the base, 1.5–2 cm. long by 1–1.3 cm. wide, obtuse or rounded at the apex, entire, truncate or subcordate at base; upper surface shining, glabrous or scabrellate, strongly bullate; lower surface closely invested with a thin gray-green tomentum; veins elevated beneath, the lateral ones ascending and confluent near the margin; veinlets prominently reticulated; upper leaves resembling the lower ones and scarcely reduced in size, bearing heads in their axils and forming several crowded cymes 10–15 cm. long; heads about 21-flowered, secund, the lower separated by internodes 1 cm. long, the upper approximate; corollas white; involucre campanulate, about 5–6 mm. high; outer scales triangular-subulate and pubescent, the inner narrowly triangular, sharply acuminate, glabrous or nearly so; achenes pubescent, 1.5 mm. long; pappus nearly white, the outer series 0.7 mm., the inner 4 mm. long.

Type, *Shafer 4176*, from pine land, altitude 400 m., along the trail from Rio Yamaniguey to Camp Toa, Oriente, Cuba, February 22–26, 1910, deposited in the Herbarium of the New York Botanical Garden.

SPECIES-GROUP SAGRAEANAE

This group is distinguished at once from other West Indian species by the large glabrous achenes. The leaves also are usually thick and firm or coriaceous, entire or with spinulose teeth. Most of the species have been in the past poorly represented in American herbaria, and some of them have been seldom collected since their original discovery.

In the revision, *Vernonia rigida* Sw. and *Vernonia fruticosa* (L.) Sw. were regarded as identical and referred to this group, to which the name *Rigidae* was applied. Since that time, specimens of *V. fruticosa* have again been collected, and the species is seen to belong to a different group. The Jamaican *V. rigida*, also, is described with pubescent achenes, a character which removes it at once from this group.

In 1836 De Candolle described *V. Sagraeana* from Cuba, the first known species of the group. This was followed in 1850 by *V. Valenzuelana* of Richard. In 1863 Schultz examined the recent Cuban collections of Wright, and added three species, *leptoclada*, *inaequiserrata* and *Wrightii*, and a fourth, *Sprengeliana*, based on a plant collected by Bertero in Santo Domingo. Grisebach added a variety, *inaequiserrata angustifolia*, also collected by Wright in Cuba. That left the group with seven species and one variety, and, so far as known to the writer, no authentic collection of any of these was made or at least recognized for forty years. Further difficulty was added by the confusion of numbers of some of Wright's collections, so that at least two different species have masqueraded in herbaria under wrong names. One case of this confusion was recognized in 1906 by Gleason, who remedied it by the description of *V. viminalis*.

Since 1906, the collectors of the New York Botanical Garden, in their diligent explorations of Cuba, have recollected four of these old, imperfectly known species, and have added three entirely new forms, which are here described.

The group as a whole is one of the most easily recognized of all the West Indian species. It is characterized especially by a high involucre and by large, glabrous, obscurely ribbed achenes, with a prominent basal callus, and the large, firm or rigid leaves.

I. Species of Santo Domingo; leaves oblong, very tomentose beneath, rugose and scabrous above, narrowly oblong, attenuate from the middle to a truncate or subcordate base; inner involucral scales rounded and apiculate at the tip. *Vernonia Sprengeliana.*

II. Species of Cuba.

A. Leaves thick, coriaceous, more or less revolute and shining above, oblong or ovate-lanceolate, remotely dentate or entire.

1. Heads 8-flowered; involucre cylindric. *Vernonia purpurata.*
2. Heads with 18 flowers or more; involucre campanulate.

a. Leaves narrowly oblong, at least three times as long as broad, entire, or with numerous minute spinulose teeth. *Vernonia Valenzuelana.*

b. Leaves broadly oblong, about twice as long as broad, entire, or with a few few remote but conspicuous teeth.

* Scales of the involucre all erect or barely spreading, the inner acute, the outer mucronate. *Vernonia leptoclada.*

** Outer scales conspicuously squarrose or reflexed, all sharply acuminate or subulate. *Vernonia Wrightii.*

B. Leaves thin or firm, but not coriaceous, flat, serrate, spinulose-denticulate, or entire.

1. Leaves puberulent or glabrous beneath.

a. Outer scales of the involucre short, acute or mucronulate, appressed. *Vernonia Sagraeana.*

b. Outer scales of the involucre elongated, subulate, spreading. *Vernonia aronifolia.*

2. Leaves tomentose beneath.

a. Middle involucral scales sharply acuminate, densely ciliate. *Vernonia viminalis.*

b. Middle involucral scales rounded to a mucronate apex, or acute.

* Inner scales obtuse or subacute; middle scales without scarious margin; leaves entire or obscurely spinulose-denticulate. *Vernonia fallax.*

** Inner scales apiculate; the middle with a scarious margin.

° Leaves narrowly linear-oblong, entire. *Vernonia ceratoides.*

°° Leaves narrowly elliptic-oblong, sharply serrate. *Vernonia inaequiserrata.*

Vernonia Sprengeliana Sch.-Bip. The type specimen cited by Schultz is *Bertero 507* and the recent description by Gleason is

based on a single sheet of *Wright, Parry, & Brummel 273*. An excellent collection of this rare species, *Fuertes 1388*, has been recently distributed and agrees perfectly with the original description and with that of Gleason (Revision, 184).

Vernonia purpurata sp. nov.

Shrubby, 2–2.5 m. tall; stem stout, coarsely striate, thinly tomentose below, becoming densely so in the inflorescence; leaves crowded, heavy, rigid, coriaceous, divaricate, elliptic-oblong, obtuse or subacute, entire or irregularly repand, obtuse or rounded at base, strongly rugose above, but glabrous and shining except for some thin pubescence along the midvein, minutely puberulent along the veins beneath; veins elevated on the lower surface, the lateral veins prominent, ascending, the veinlets small and closely reticulated; petiole 2–4 mm. long, tomentose; inflorescence small, irregular, composed of several short (2–6 cm.) leafy cymes, bearing each 4–10 heads; rameal leaves resembling the cauline, but two thirds as long; bracteal leaves narrowly oblong or oblong-linear, 10–15 mm. long, not present below many of the heads; heads sessile, secund along the cymes or aggregated at their tips, 8-flowered; corollas white; involucre narrowly cylindric, 6 mm. high; scales closely imbricated, appressed, sharply acute, the lower ovate-triangular, pubescent, the middle ones with an ovate-triangular exposed portion, ciliate, glabrous on the back, the inner entire, puberulent on the back, purple-brown at their exposed tips; achenes glabrous, immature in the type specimen; pappus pale yellow-brown, the outer series 1.3 mm., the inner 7 mm. long.

Type, *Taylor 544*, from Jiquarito Mountain, Sierra Maestro, eastern Cuba, altitude 1,020 m., September 18, 1906, deposited in the Herbarium of the New York Botanical Garden.

The lower leaves are lacking from the type plant. The crowded upper leaves are remarkably uniform in size, 4–5 cm. long by 1.5–1.9 mm. wide. On another branch is the base of a leaf which measures 3 cm. wide, indicating that the lower leaves are considerably larger than the upper. While the species certainly belongs to this group, it is distinguished from all the others known by its few-flowered heads.

Vernonia Valenzuelana Rich. A shrub 1.2 m. high, on dry ferruginous soil, southeast of Paso Estancia, Oriente, Cuba, *Shafer 1705*.

Vernonia leptoclada Sch.-Bip. A specimen agreeing perfectly with the original description has been recently collected by Shafer, *8145*, from Camp La Gloria, south of Sierra Moa, Oriente, Cuba. It is described as a straggling shrub 3.5 m. tall, with white flowers.

Vernonia Wrightii Sch.-Bip. *Ex descr.* A shrub, 1 m. high, freely branched above; stems pubescent; leaves heavy and coriaceous, spreading or ascending, ovate-oblong to oblong, 3.5 cm. long by 1.2 cm. wide, sharply acute or mucronate, revolute, entire or with a few remote spinose teeth, rounded or even subcordate at the base, very scabrous above but not pubescent, minutely puberulent under the lens beneath; petioles 1–2 mm. long, pubescent; cymes long and spreading, with numerous heads; bracteal leaves like the cauline, but gradually reduced to 1 cm. long; heads sessile, secund, about 21-flowered; involucre about 7–8 mm. high, outer scales ovate, acuminate into a squarrose or recurved tip, the inner erect or somewhat spreading, sharply acute or subulate; pappus pale brown.

Shafer 7738, from a dry serpentine hill near El Yunque, Oriente, and *Shafer 3072*, from pine lands at 500–650 m. altitude near Woodfred, Oriente, are referred here. They agree in most essential points with Schultz' description of *V. Wrightii* (Journ. Bot. 1: 234. 1863), but lack the glabrous leaves narrowed at both ends and the sordid-purple pappus.

Schultz' species is based on *Wright 1309*. As is well known, the Wright numbers are much confused, and frequently shelter more than one species. The only available specimen of this number belongs to an entirely different species.

Vernonia Sagraeana DC. A shrub 1.5 m. tall, growing on banks at an altitude of 325 m., near El Cuero, Oriente, *Britton & Cowell 12794*.

Vernonia aronifolia sp. nov.

Bushy, suffrutescent or shrubby, 12–15 dm. high; stem stout, freely branched, striate, finely and thinly tomentose, the tomentum increasing in density toward the tips of the branches and always most abundant in the axils; leaves dark green, thin but firm, obovate-oblong, about 8 cm. long by 4 cm. wide, abruptly acuminate, remotely denticulate with subulate or spinulose teeth 0.5

mm. long, standing at right angles to the margin, obtuse or subacute at base, minutely puberulent beneath, especially along the veins, glabrous above; veins prominent, light green, the lateral ones arcuate-ascending, branched and reticulated, especially toward the margin, one of the smaller marginal veins prolonged into each denticulation; cymes terminal and lateral, spreading, unbranched, 15–20 cm. long, very leafy, bearing 6–10 heads; bracteal leaves resembling the cauline in shape and texture, or varying to broadly oblong-elliptic in shape; the lower approximating the cauline in size, the upper gradually reduced to half the size, but always greatly exceeding the flowers; heads sessile, single in the axils, about 34–47-flowered; corollas white; involucre hemispherical or broadly campanulate, 8–9 mm. high, in dried specimens about 15 mm. broad; scales thinly puberulent, imbricated only at the base, straight or erect in bud, becoming spreading or flexed in fruit, the outer lance-linear, subulate, the inner linear-oblong, acuminate to a subulate tip; achenes smooth; outer pappus white, 0.8 mm. long, inner series pale brown or nearly white, 8 mm. long, minutely barbellate.

Type, *Shafer 13514*, collected from high rocks in limestone hills, vicinity of Sumidero, Province of Pinar del Rio, Cuba, August 2, 4, 1912, deposited in the Herbarium of the New York Botanical Garden. The collection is represented by two sheets, one including apparently the top of the plant, and the other two detached lateral branches.

Vernonia fallax sp. nov.

Shrubby, 1 m. high; stem erect, sparingly branched, finely striate, closely gray-tomentose, especially above; leaves firm, bright green above, elliptic-oblong, 5–7 cm. long by 2–2.5 cm. wide, acute, mucronate, entire or very obscurely and remotely spinulose-denticulate, narrowed to an acute base, thinly puberulent above, especially on the veins, finely and closely gray-tomentose beneath; veins elevated and prominent beneath, not conspicuously reticulated; petioles 1–2 mm. long; inflorescence pyramidal, terminal, of about 4–10 short, spreading or recurved cymes bearing each 3–7 heads; heads secund, sessile, about 21-flowered; involucre broadly campanulate or subhemispherical, 6–7 mm. high, its scales appressed, closely imbricated, pubescent, especially near the tip, outer scales ovate-triangular, acute and cuspidate, the inner subacute or rounded at the tip; outer pappus white, 0.7 mm. long, the inner pappus very pale brown, 6 mm. long; achenes glabrous.

Type, *Britton & Wilson 5478*, from a hillside, altitude 500 m., in the Trinidad Mountains, Province of Santa Clara, Cuba, March 12, 1910, deposited in the Herbarium of the New York Botanical Garden.

The bracteal leaves have all fallen off the type specimen, except a few fragments, and the leaves are crowded on short lateral branches. The inflorescence is thus left at the end of a naked peduncle 2–3 dm. long, giving the specimen an aspect entirely unlike other species of the group. It is scarcely to be expected that the same peculiarity will be maintained in other collections of the species.

Vernonia aceratoides sp. nov.

Vernonia inaequiserrata angustifolia Griseb. Cat. Pl. Cuba 144. 1866.

Slender and probably herbaceous; stem finely striate, closely gray-tomentulose; leaves firm, spreading or ascending, narrowly oblong-lanceolate or lance-linear, the principal ones 7–8 cm. long and 1–1.2 cm. wide, acute and mucronulate at the tip, entire or somewhat repand; obtuse or rounded at the base, minutely scabrellate above and puberulent along the midvein, finely brown-tomentulose beneath; veins prominent beneath and conspicuously reticulated; petioles 2–3 mm. long; inflorescence terminal, of about 3 short divaricately spreading cymes, bearing each six or seven secund heads; bracteal leaves oblong, the upper ones not exceeding the heads, and all proportionately broader than the cauline; involucre narrowly campanulate, 5–6 mm. high; scales closely and regularly imbricated, appressed, the outer ovate-triangular, cuspidate, the inner with an ovate exposed portion, rounded and apiculate at the tip.

Grisebach's variety was based on a specimen of *Wright 2784;* the preceding more detailed description is based on a sheet of the same number in the Herbarium of the Missouri Botanical Garden.

SPECIES-GROUP LONGIFOLIAE

The herbarium of Dr. Otto Kuntze contained a good specimen of a *Vernonia* from St. Thomas, collected by Kuntze himself in 1874, and labeled *Vernonia Thomae* Benth. It can not be distinguished, however, in any essential character from *Vernonia albicaulis* Pers., and the two species may henceforth be considered identical. This disposition of *V. Thomae* was suggested before by Gleason (Revision, 191), although at that time the two were

kept separate since good material for examination was lacking. The island of St. Thomas is accordingly added to the known distribution of *V. albicaulis*. It has been collected in St. Thomas by others also, and is represented in the herbarium of the Field Museum by two sheets, *Millspaugh 522* and *Eggers 34*.

Vernonia longifolia Pers. To the distribution of this species may be added St. Martin, *Boldingh 2641*, and Montserrat, *Shafer 172, 589, 659, 661*.

Lepidaploa, *Scorpioideae reductae*

Vernonia arctata Gleason. The species was originally described (Bull. Torrey Club **33**: 185. 1906) from New Providence Island, of the Bahama group, but is now known to occur also throughout Andros Island, *Small & Carter 8506, 8613, 8759, 8890, Brace 5176, 6754, 6926, 7138*. Field data show that the flowers vary from purplish white to bright rose-purple and that the plant reaches a height of 2 meters.

Vernonia bahamensis Griseb. Reported by Gleason (Bull. Torrey Club **33**: 187. 1906) from Fortune Island and Inagua, it is now represented also by specimens from Crooked Island, *Brace 4851;* Acklin's Island, *Brace 4330;* Salt Cay, *Millspaugh & Millspaugh 9249;* Long Cay, *Brace 4152, 4020, 4115;* Mariguana, *Wilson 7461;* Castle Island, *Wilson 7783;* Cotton Cay, *Millspaugh & Millspaugh 9362;* North Caicos, *Wilson 7721, Millspaugh & Millspaugh 9175;* East Caicos, *Millspaugh & Millspaugh 9082;* and South Caicos, *Wilson 7688*. The last specimen cited has leaves subacuminate or merely acute at the base, 5 cm. long by 2.5 cm. wide, and in general closely approximates *V. albicaulis* Pers. The *Scorpioideae reductae* have been considered (Gleason, Revision, 165, 166) as related by origin to the species-group *Longifoliae*, to which *V. albicaulis* belongs, and the theory is strengthened by the strong superficial resemblance just mentioned. It is interesting to note that *V. bahamensis* occupies the southeastern portion of the Bahama archipelago, nearest the area of the *Longifoliae*, and that the particular specimen comes from South Caicos, which is almost the extreme southeastern island of the group.

Vernonia complicata Griseb. In the type collection, *Wright 2790*, the leaves are all entire, subrotund, and about 5 mm. long.

An excellent specimen, *Britton 2225*, recently received at the New York Botanical Garden from Guantanamo Bay, in extreme eastern Cuba, has leaves of the same character on the old shoots, while on the young branches they are flat or undulate and 10–15 mm. long. The Guantanamo plant is described as a shrub 1 m. tall with purple flowers.

Lepidaploa, *Scorpioideae aggregatae*

Vernonia Thomae Benth., included here by Gleason (Revision, 191), is now regarded as identical with *Vernonia albicaulis* Pers. Urban (Symb. Antill. 7: 421. 1912) has recently added a species, so that the number in the group remains four. They may be distinguished as follows:

A. Achenes pubescent; outer pappus conspicuous, its scales much broader than the white bristles of the inner series; leaves 2–3 cm. long. *Vernonia buxifolia* (Cass.) Less.

B. Achenes glabrous and glandular; outer pappus minute, its scales not sharply distinguished in width from those of the inner series.

1. Leaves tomentulose beneath; pappus strongly tinged with rose color; heads about 11-flowered. *Vernonia Tuerckheimii* Urban.
2. Leaves minutely puberulent or glabrous beneath; pappus yellowish or tawny; heads 8-flowered. *Vernonia montana* Gleason.

C. Achenes densely hirsute; outer pappus conspicuous, its scales chaffy and fimbriate; leaves 4–5 cm. long, closely gray-tomentose, and with prominent veins beneath. *Vernonia yunquensis* Gleason.

The first three species are all very similar in habit and structure, and are all natives of Hispaniola. There is no doubt that they are closely related. The character of the pappus and the larger leaves indicate that *V. buxifolia* is the primitive form. The distribution (Cuba) and the general habit of *V. yunquensis*, especially of the leaves, as expressed in pubescence and venation, separate it sharply from the first three species, and imply that it may logically constitute another species-group.

Vernonia segregata sp. nov.

A straggling or vinelike shrub, reaching a height of 2.5 m.; stem obscurely striate, closely pubescent; the branches olive-

brown and finely tomentulose; leaves numerous, crowded, firm, dark green, oblong to elliptic-obovate, broadest at or above the middle, the principal ones 2.5–4 cm. long and 1–1.5 cm. wide, obtuse or subacute, entire, obtuse or rounded at the base, scabrellate and resinous-punctate above, glabrous beneath and densely punctate with resinous globules and impressed black glands; midvein puberulent, the lateral veins inconspicuous; petioles 1–2 mm. long; inflorescence terminal, irregular in shape, consisting of several short, simple or sparingly branched cymes 2–4 cm. long, naked below, and bearing 2–8 crowded heads in a terminal subcapitate cluster, or at the base of the branches; bracteal leaves 1–3 subtending each cluster of heads, resembling the cauline in shape, 5–15 mm. long; heads about 8-flowered; corollas white; involucre campanulate, 3–4 mm. high, its scales loosely and irregularly imbricated, appressed at the base, but spreading at the tip, stiff and firm in texture, the outer narrowly triangular-lanceolate, long-acuminate, the inner narrowly oblong-linear, tapering gradually to the acuminate puberulent apex; achenes thinly pubescent, 2 mm. long; pappus nearly white, the outer series 1 mm., the inner 4 mm. long.

Type, *Shafer 4050*, from rocky river banks in the vicinity of Camp San Benito, Oriente, Cuba, altitude 900 m., February 24, 1910, deposited in the Herbarium of the New York Botanical Garden. Other sheets in the same herbarium, all collected in the mountains of Oriente, are *Shafer 8051*, stated to be vinelike and 8 feet high; *Shafer 8216*, 1.5–2 feet high; and *Shafer 4446*, described by the collector as an herb three feet high with purple flowers. Notwithstanding field differences in the color of flowers or texture of stem, all four numbers clearly belong to the same species.

The relationship of *V. segregata* is puzzling. The subcapitate clusters clearly represent a modification of a scorpioid type, and most closely resemble the inflorescence of the *Scorpioideae aggregatae*. The involucre is quite different, however, from that of typical members of the group. For the present, it has been considered advisable not to assign the species to any group.

SPECIES-GROUP HAVANENSES

In recent work on *Vernonia*, *V. havanensis* and *V. Ottonis* have been considered identical (Revision, 192). The large series of specimens now available for study permits the ready separation of two species, with characters so typical that to each can be

assigned the proper specific name without difficulty. In addition, a new species has been collected by Shafer and is described below.

With the exception of *V. pallescens*, whose position in this group is somewhat uncertain, the group is distinguished by similarities in habit. The leaves are of a comparatively broad type, widest near or usually above the middle, and with the serration most prominent on the distal half. The involucre scales are regularly pubescent in two areas on the back, one on each side of the mid-nerve.

The four species may be distinguished as follows:

A. Inflorescence strictly scorpioid; the cymes many-headed and elongated; heads all sessile. *Vernonia pallescens.*

B. Inflorescence freely branched and subpaniculate, some of the heads pedicellate; leaves with a tendency to be broadest above the middle; scales glandular on the back, especially on each side of the mid-nerve.

1. Heads with 18 flowers or more; involucres 5–8 mm. high, or some of the scales 10 mm. long, distinctly purple-tinged; leaves essentially glabrous on both sides; pappus white, or with a faint brownish yellow tinge. *Vernonia havanensis.*

2. Heads 5–13-flowered; involucres 3–4 mm. high, obscurely or not at all tinged with purple; leaves scabrous above; pappus pale brown.

a. Heads 11–13-flowered; inner scales obtuse or subacute; inflorescence divaricate. *Vernonia Ottonis.*

b. Heads 5-flowered, aggregated in subcapitate clusters at the ends of the branches, forming a pyramidal or subhemispheric inflorescence; inner scales acute. *Vernonia Orientis.*

Vernonia pallescens Gleason. The species certainly differs phylogenetically from the rest of the group, as shown by its inflorescence and its geographical distribution. It is included in the group merely for lack of a better place to put it.

VERNONIA HAVANENSIS DC. Prodr. 5: 37. 1836.

Vernonia stictophylla Wright, Sauv. Anal. Acad. Ci. Habana 6: 176. 1869.

The specimens at hand fall into two groups, the first with leaves long-attenuate at base, almost sessile, and thin in texture; the second with leaves cuneate into a distinct petiole and firm in texture. No other characters for their separation have been

found. The first group includes the type collection for Wright's species. Both series have been collected, so far as data are given, in the province of Pinar del Rio.

VERNONIA OTTONIS Sch.-Bip. Linnaea **20**: 508. 1847.

Vernonia hieracioides Griseb. Mem. Am. Acad. **8**: 511. 1860.
Vernonia cubensis Griseb. Cat. Pl. Cuba 144. 1866.

Except one collection from Santa Clara (*Leon 1315*) and one from the Isle of Pines (Curtiss), all the sheets examined are from Pinar del Rio. They show considerable variation in the pubescence, serration, and texture of the leaves, but can not be further separated. The specimens include cotypes of both Grisebach's species, and agree perfectly with Schultz' description.

Vernonia orientis sp. nov.

Shrubby, as much as 6 m. in height, apparently not extensively branched; stem coarsely striate, glabrate below, becoming cinereous-puberulent in the inflorescence; leaves rigid, dark green, spreading, oblanceolate, the principal ones 9–11 cm. long by 2.5–3.5 cm. wide, abruptly short-acuminate or sharply acute, remotely dentate with sharp salient teeth, chiefly above the middle, attenuate from below the middle to a cuneate base, very scabrous above, minutely puberulent and scabrellate beneath; veins elevated below, only the midvein and its lateral branches prominent; petioles 5–10 mm. long; inflorescence terminal, broadly pyramidal or subhemispheric; cymes freely branching, ultimately bearing 2–6 heads aggregated or subcapitate near the tips; bracts subulate, 3–5 mm. long; heads 5-flowered; involucre 3–4 mm. high, campanulate; scales ovate to ovate-oblong, sharply acute or subacuminate, essentially glabrous but glandular on the back; achenes sparingly pubescent; outer pappus minute, the inner pale yellowish brown, 4 mm. long.

Type, *Shafer 3509*, from Sierra Nipe, near Woodfred, Oriente, Cuba, altitude 450–550 m., January 10, 1910, deposited in the Herbarium of the New York Botanical Garden.

The two collections, both from Oriente, are the only examples of the group from this part of the island. It is distinguished from the other members of the group at a glance by its inflorescence, and also by the involucral scales and the number of flowers.

Lepidaploa, *Paniculatae dichotomae*

VERNONIA MENTHAEFOLIA (Pöpp.) Less. Linnaea 4: 268. 1829.
Eupatorium menthaefolium Pöpp. in Spreng. Syst. 3: 412. 1826.
Vernonia Grisebachii Sch.-Bip. Jour. Bot. 1: 231. 1863.

The original description of this species by Pöppig is too brief to be of any value at the present time. But his specimens were preserved, and examined later by both Schultz and Lessing. Lessing gives a detailed description, based on these types, stating that the heads are many-flowered and 3 lines high. Schultz' description, referring without doubt to the same specimens, or to duplicates of them, indicates that the heads are 11-flowered and the involucre hardly 1 line high. He then described *V. Grisebachii*, as cited above, to include the forms with large heads, based on *Wright 1305*. Examination of an ample series of specimens at the present time reveals but one species, agreeing with Lessing's and Schultz' descriptions, but never with the small heads ascribed by the latter to *V. menthaefolia*. In the series examined are two of Wright's collections, *282* and *2792*, and *Shafer 8811*, which was found by Dr. Britton to agree with the specimen of *Wright 1305* in the Kew herbarium. Throughout the series the heads have 11 to 18 flowers, and the involucres are 4–5 mm. high. The leaves show considerable variation, from narrowly oblong-lanceolate, acuminate at both ends, to ovate, rounded at the base and acute at the apex. These characters are not sufficiently definite or constant to permit the recognition of two species.

V. menthaefolia is the most abundant species of the genus in Cuba, judged from the frequency of its collection, and occurs throughout the island.

Among recent accession to the Herbarium of the New York Botanical Garden is an *Eremosis* from the state of Durango, which differs distinctly from all the fifteen described species of the genus.

Eremosis ovata sp. nov.

Shrubby; height and habit not stated; stem obscurely striate, closely cinereous-pubescent, becoming tomentulose in the inflorescence; leaves thick, firm, ovate to ovate-elliptic, 7–10 cm. long, 4–5 cm. wide, obtuse or subacute, entire, obtuse at base, dull green, minutely and softly tomentulose above, densely cinereous-

tomentose beneath; veins elevated below, the lateral ones prominent and ascending, the veinlets inconspicuous; petiole 8–13 mm. long; inflorescence broadly pyramidal or hemispheric, about 2 dm. wide; rameal leaves elliptic, about 2–6 cm. long, otherwise like the cauline; heads 4-flowered, in clusters of 3–8, on pedicels 2–5 mm. long; involucre narrowly campanulate, straw-colored or pale brown, 5–6 mm. high, outer scales short, broadly ovate, obtuse to subacute and apiculate, irregularly arachnoid or tomentulose, inner scales deciduous, oblong or ovate-oblong, acute, glabrous, or with minute patches of thin tomentum near the tip; achenes pale brown, 3 mm. long, prominently ribbed, thinly hirsute with ascending hairs; pappus white, 8 mm. long, the outer series much shorter.

Type, *Palmer 139*, from San Ramon, Durango, Mexico, deposited in the Herbarium of the New York Botanical Garden.

In general habit and shape of leaf, *Eremosis ovata* most closely resembles *Eremosis Steetzii* (Sch.-Bip.) Gleason, but is distinguished at once from this one-flowered species by its four-flowered heads. Its nearest relatives are probably to be found among the three-flowered species, such as *Eremosis Palmeri* (Rose) Gleason, from which it differs in the broad ovate leaves and dense tomentum.

The presence regularly of four flowers in each head is a peculiar feature, hitherto unknown in the genus. It is paralleled in a way, however, by the occurrence of two flowers instead of one in the heads of certain specimens of *Eremosis tarchonanthifolia* (DC.) Gleason.

UNIVERSITY OF MICHIGAN, ANN ARBOR.

Some toxic and antitoxic effects in cultures of Spirogyra *

W. D. Hoyt

In view of difficulties commonly experienced in maintaining algal cultures in the laboratory, it seemed desirable to make an experimental study of some of these difficulties, as was suggested to the writer by Professor Georg Klebs, and the present publication deals with a portion of such a study. The experimentation was carried out in the Botanisches Institut at Heidelberg in 1909 and 1910, the facilities of these laboratories being made available through the kindness of Professor Klebs. The experiments to be considered below bear upon the preparation of a nutrient solution suitable for algal growth under laboratory conditions, and undertake a partial analysis of the relations of the aqueous medium to the success or failure of algal cultures in glass.

The alga used for this study was *Spirogyra longata* (Vauch.) Kg.,† brought from Algiers in the spring of 1906 and kept since then (until the winter of 1909–10) in a north window of the Botanisches Institut at Heidelberg, with only small additions of tap water from time to time. At the time of beginning these studies the culture was healthy and showed vigorous growth, and the material seemed excellently suited for experimentation of the sort in hand; all the filaments had developed under closely similar conditions and were obviously adjusted to the conditions existing in the laboratory. In the experimentation it was thus possible to alter the culture medium without greatly changing the other environmental factors—*e. g.*, light, temperature—under which the plants had developed. The last consideration is an important

* Botanical Contribution from The Johns Hopkins University, No. 29.

† Determined from Einfachste Lebensformen des Tier- und Pflanzenreiches, Eyferth, B., 3 Aufl. 1900.

one; results obtained with material brought from various other conditions to the laboratory cannot be interpreted as caused by changes in the nature of the culture solution alone, a point well emphasized by some of the considerations to be brought out below.

The method here followed was to transfer a few (about 30 to 50) filaments from the stock culture to about ten cubic centimeters of the medium to be tested, in a covered glass dish. These transfers were made late in the afternoon, and the cultures were subjected to microscopic examination, without removal from the culture dishes, on the next morning and on succeeding days until final results were obtained. The experiments lasted for periods of from one to eighty-six days. The condition of each culture was recorded in the following terms. *Excellent* means that, at most, only a few cells showed injury; *good*, that considerably more than half of the filaments seemed uninjured; *poor*, that more than half were injured; *dead*, that practically every cell was dead. The tables used in this paper have been constructed from the daily records by stating in similar terms the general condition of the cultures throughout the experiments.

Precautions were of course taken to have the dishes and instruments as clean as possible. Any dish once used for a poisonous solution was discarded at the end of that experiment, and dishes were sometimes interchanged and furnished with fresh supplies of the media to be tested. The different lots of special distilled water did not enter into experiments until they had been found to be harmless to the plant here used. In most cases about the same amounts of media were used in the different experiments; the quantity of solution as related to the number of cells in any culture is to be considered as an important factor in all experiments bearing upon the relation of the nature of the medium to the behavior of organisms existing therein.

I. NUTRIENT SOLUTIONS

The nutrient media used were all prepared according to the formulae given by Küster (10), being those of Sachs, Knop, Molisch, and Crone.* These were afterward diluted to the

* These solutions contained salts in the following proportions:

required concentration with either ordinary or nontoxic distilled water. The reactions to litmus paper of the solutions thus prepared were as follows: Sachs's, very slightly acid; Knop's, acid; Molisch's, slightly acid; Crone's, neutral. The salts used were, in every case, those of Merck.

The results obtained from the various cultures in these nutrient solutions are given in TABLE I, where each culture is denoted by a letter indicating the condition of the culture during the period of the experiment, the number of letters thus denoting, in each case, the number of times that the experiment in question was performed. The durations of the cultures are also given. The same notation is used in TABLES II, III, and V.

Using nontoxic distilled water, prepared in a manner to be described later, the best growth was shown with all the media in concentrations containing from 0.05 to 0.1 per cent of total salts. In these concentrations of the solutions of both Molisch and Crone, *Spirogyra* was kept growing vigorously, in apparently perfect condition, for a period of about two months, at the end of which time the experimentation was discontinued. Crone's solution seemed slightly more favorable to the organism than did that of Molisch; Sachs' solution produced a fair growth; while Knop's medium was distinctly unfavorable, probably because of the marked acidity, free acid being injurious to many algae (10). No doubt still other media might prove as favorable as those of Molisch and Crone.

When ordinary distilled water was used in preparing the solutions a good growth was never obtained, but the results in both Molisch's and Crone's solutions seemed slightly better in concentrations of from 0.5 to 1 per cent than in weaker solutions. This will be discussed later.

The addition of one per cent of agar to the nutrient solutions, the *Spirogyra* filaments being laid on the surface of the coagulated

SACHS	KNOP	MOLISCH	CRONE
1 g. KNO_3,	1 g. $Ca(NO_3)_2$,	1 g. $(NH_4)_2HPO_4$,	1 g. KNO_3,
0.5 g. NaCl,	0.25 g. KNO_3,	0.5 g. KH_2PO_4,	0.5 g. $MgSO_4$,
0.5 g. $CaSO_4$,	0.25 g. $MgSO_4$,	0.5 g. $MgSO_4$,	0.5 g. $CaSO_4$,
0.5 g. $MgSO_4$,	0.25 g. KH_2PO_4,	0.5 g. $CaSO_4$,	0.25 g. $Ca_3(PO_4)_2$,
0.5 g. $CaHPO_4$,	0.125 g. KCL,	Trace $FeSO_4$.	0.25 g. $Fe_3(PO_4)_2$.
Trace $FeCl_2$.	Trace $FeCl_2$.		

medium in a covered Petri dish, produced slight improvement in all the 0.1 per cent solutions from ordinary distilled water. A good growth was obtained on similar agar plates prepared from 0.5 per cent solutions (in ordinary distilled water) according to the formula of Sachs, of Molisch, and of Crone.*

Since the solutions here employed were all prepared from water and salts, it will be expedient to present the following considerations under the two headings: (II) Water and (III) Salts.

II. WATER

Many authors have found that tap water and distilled water are toxic to organisms, an excellent review of the literature of this subject being given by Livingston (12). Most workers have attributed the toxic effects of ordinary distilled water to small amounts of various metals—especially copper—taken by the water from the still and from the supply pipes. Lyon (19) and Bullot (6), however, found that water distilled in glass was markedly toxic, thus indicating that part of the poisonous action may be due to the presence of volatile substances. Lyon attributed this effect to small quantities of ammonia in the water. Similarly Livingston (12) showed that redistillation from glass to glass, while improving the quality of his ordinary distilled water, did not render it harmless. He obtained evidence indicating that part of the toxic bodies present in ordinary distilled water thus redistilled are volatile and reappear in the distillate, while part are nonvolatile and remain in the undistilled residue. The same writer prepared nontoxic water by shaking ordinary distilled water with highly absorbent solids, especially with purified lamp black, and then filtering out the solids. He concluded that the solids had absorbed, or at least removed from solution, both the volatile and the nonvolatile toxic substances.

In the present investigation both tap water and ordinary distilled water were found to be markedly toxic to *Spirogyra*, and were subjected to various treatments in an effort to obtain some evidence concerning the nature of the poisonous substances involved. The tap water was drawn from the faucet of the laboratory

* Knop's solution was so acid that 1 per cent agar would not harden in a 0.5 per cent concentration of the medium.

supply, directly into the culture dishes. The distilled water used was from the usual supply of the laboratory, obtained from a local druggist. It had been distilled in a copper still and kept in glass. All such water used in these experiments was from the same original supply and may thus be considered as uniform. The results obtained in the study of the physiological properties of various waters are given in TABLES II and III.

Tap water was usually fatal to the plant within a few days, although its toxicity varied slightly at different times (II, 1*). This water, when distilled with glass boiler and glass condenser, was as toxic as the untreated water (II, 2). The distillate was then sampled at different times during the run, by placing culture dishes at the outlet of the condenser and allowing them to receive the water directly; the alga was introduced into these water samples after they had cooled. Samples collected at the beginning and end of the run were as toxic as the untreated water (II, 3, 3*b*). About the middle of the run the distillate collected was found to be decidedly less injurious than the untreated tap water (II, 3*a*). Concentration of the tap water to one tenth its original volume by boiling (II, 4) and also by allowing evaporation to proceed at a temperature much below boiling, till only about a sixth of the original volume remained (II, 5, 6), produced marked improvement over the original untreated water, but concentration by evaporation to one third of the original volume did not improve its quality (II, 7). It will be noted, however, that the untreated water of this particular test was more toxic than in the two former experiments. In general, concentration seems to have improved the quality of the water, probably by driving off some of the volatile toxic substances, though it is of course not to be forgotten in this connection that the nonvolatile constituents were much more concentrated in the treated water than in the original.

The effect of high temperature without concentration was also tested. Heating tap water in the autoclave for 15 minutes, at a temperature of 144° C., destroyed all the toxic material, or else rendered it nontoxic; in water thus treated *Spirogyra* filaments remained in perfect condition for a month, while in untreated

* Roman numerals followed by Arabic, in parentheses, refer to table and experiment, respectively.

water drawn from the tap at the same time, nearly all the filaments succumbed within a few hours (II, 8). Heating tap water to a temperature of 100° C. in a steam sterilizer for 45 minutes produced no improvement; the filaments of *Spirogyra* died almost as quickly in this as in untreated water (II, 9).

Treatment of the tap water of the Heidelberg supply by the method of shaking with finely divided carbon, as employed with such marked effect by Livingston in the preparation of culture media from his ordinary distilled water, produced no improvement. In this treatment Merck's "animal charcoal" was employed as absorbing solid, without preliminary treatment of any sort. While it seems probable that there may be essential differences between the various forms of finely divided carbon now upon the market, as to their efficiency in the water treatments here considered, no comparisons have been made in this regard. The form here used was chosen merely because of its convenience.

Although, as has been pointed out, simple distillation of the Heidelberg tap water from glass into glass failed to correct the toxicity of the water, it was found that this same process of distillation rendered the water nontoxic to the *Spirogyra* here employed (II, 10) *when animal charcoal was present in the retort during the distillation*. In this treatment the boiler was a flask of Jena glass having a capacity of one liter; the condenser was of glass, provided with a bend to prevent the entrance of spray from the boiling liquid and thoroughly washed by use; and the receiver was a flask of Jena glass. No cork or rubber or material other than glass was used on the condenser. The usual charge was about 800 cc. of tap water with from five to ten grams of Merck's animal charcoal. Boiling was not allowed to become very violent and the process was stopped when the charge had been reduced to about one eighth of its original volume. In water thus prepared *Spirogyra* filaments remained in apparently healthy condition until the appearance of injury from lack of nutrient salts.

This method of distillation from animal charcoal showed some variation in the quality of the water produced (II, 10), but the differences exhibited were not as great as those obtained with different portions of water from the stock culture (III, 36). The water distilled from charcoal was frequently tested throughout

the experiments,—by determining its effect, when used alone, upon *Spirogyra* filaments. Out of more than ten runs the distillate from but a single one was found to be decidedly toxic to the alga. The cause of this single failure was not investigated. The new method was uniformly employed for the preparation of nontoxic water throughout the course of these studies.

Several experiments were carried out to throw light upon the question of the manner of operation by which distillation from animal charcoal may produce such a marked effect upon the physiological properties of the Heidelberg water. Not only the distillate but also the undistilled and concentrated residue (after removal of the charcoal by filtration through paper) showed marked improvement as compared with the untreated tap water (II, 11, 12). Furthermore, a high degree of toxicity was produced in the practically nontoxic water, either of the filtered residue or of distillate, by the addition of a portion of the used charcoal that had been retained in the filter (II, 11*b*, 12*a*, 12*c*).

From the above observations it appears clear that the animal charcoal in the boiler of the still must have acted to remove toxic substances from the water, although, as has been stated, such action could not be detected at ordinary temperatures. It also appears that charcoal which has been the agent in this removal of injurious material becomes itself able to reproduce toxic properties in nontoxic water to which it may be added. It may be therefore considered as at least highly probable that the action of the finely divided carbon of these distillation experiments was that of an absorber rather than that of an oxidizing or other catalytic agent. Of course it is open to question, whether the toxicity reproduced in harmless water by used animal charcoal may be caused by the same substances as was the original toxicity of the tap water before treatment. The state of our knowledge of these matters precludes their further analysis at this time.

One experiment was made to show the relative resistance to the toxic influence in tap water of two portions of *Spirogyra* which were originally from the same source but which had been subjected for a time to different conditions. One sample was taken from the stock jar and another from a vigorous culture which was originally from the stock culture and then had grown for the last 68

days in 0.1 per cent Crone's solution. The two samples contained approximately equal numbers of filaments. They were well rinsed in nontoxic water and then placed together in untreated tap water in a covered glass dish. Within a few hours the filaments from the stock jar showed decided injury, and, at the end of six days, when the experiment was discontinued, about one third of them were dead (II, 13). The filaments transferred from Crone's solution showed no signs of injury during the period (II, 14). This experiment emphasizes the facts, well recognized, but not always considered by workers with cultures, that the internal conditions of organisms are fully as important in determining their behavior as are the conditions of the surroundings, and that the past history of organisms (the sort of surroundings under which they *have* lived) greatly influence their internal nature. Comparable results cannot be expected, in such work as this, unless the living material used has been kept under uniform conditions for a considerable time previous to experimentation.

Turning to the results obtained with ordinary distilled water (TABLE III), this was found to be extremely toxic, killing all the filaments of *Spirogyra*, except in a single instance,* in from one to nine days (III, 1). Redistilling this water in glass produced a decided improvement in some cases, but none in others (III, 2–6). Similarly, the samples of water obtained as above described, at the beginning, middle and end of the distillation run, varied greatly and inconsistently among themselves. The undistilled portion left in the boiler after redistillation had been stopped was always just as toxic as untreated water (III, 2*a*, 3*c*, 4*e*, 5*e*). Making from the distillate or from the undistilled residue (III, 4, 5) a 0.5 per cent concentration of Crone's solution produced no improvement, so that this addition of nutrient salts failed to counteract the toxicity of these two waters. The addition of animal charcoal to the boiler during redistillation in glass usually produced no improvement over the water redistilled without the solid (III, 7, 8). Furthermore, the undistilled residue from such redistillation (after removal of the carbon) showed no marked improvement. Boiling with or without carbon (III, 2–10) is thus seen to have produced

* In this case an unusually small amount of distilled water was used, in proportion to the number of filaments in the culture.

no correction of the toxic conditions of ordinary distilled water. Heating in an autoclave for 15 minutes at 144° C. made ordinary distilled water decidedly less injurious, but did not render it entirely nontoxic (III, 11).

Dilution with an equal volume of physiologically pure water (III, 12) or with water from a vigorous culture of *Nitella* (III, 13) did not make the ordinary distilled water less toxic, but dilution with an equal volume of a colloidal platinum solution (III, 14) rendered this water less injurious.*

The addition to ordinary distilled water, at the time of adding the alga, of filter paper (III, 15), cotton (III, 16), kaolin (III, 17), quartz sand (III, 18), or small amounts of chalk (III, 19, 20), produced no improvement; but the addition of abundant chalk (III, 21), lime (III, 25), broken agar sticks (III, 27), or dry sphagnum moss (III, 31) usually rendered the water nontoxic. *Spirogyra* filaments died within a few hours in ordinary distilled water to which a small amount of agar solution was added (III, 28), they lived nearly two months on the surface of a 1 per cent. solution of agar made with this same water (III, 29), but died within three days on the surface of a 2 per cent agar solution (III, 30). In ordinary distilled water shaken with chalk (III, 22), wood charcoal (III, 23), or garden soil (III, 24) and decanted after the subsidence of the solid, little or no improvement was observed at first, but decided improvement was shown after a few days. Addition to the water of sediment from a vigorous culture of *Nitella* (III, 32) produced better growth than that occurring in untreated water, while sediment from the stock culture of *Spirogyra* (III, 33) produced no improvement.

It was found that the alga filaments themselves may exert a profound influence upon the toxicity of the solution in which they are immersed. A transfer of filaments was made, in the usual manner, from the stock culture to a dish of ordinary distilled water (III, 34). These filaments were dead within eighteen hours. They were then removed from the culture and replaced by a new transfer of filaments from the stock culture. These also died within eighteen hours, and were replaced as before. The third

* This colloidal platinum solution was kindly prepared by Dr. W. Fraenkel in the laboratory of Prof. G. Bredig. It contained 0.0096 g. of platinum per 100 c.c.

transfer was killed in about two days, and the material which then replaced it lived fifteen days, but was in apparently poor condition at the end of that time and was replaced. The material of this fifth transfer preserved an apparently healthy condition for forty-four days, at which time the experiment was discontinued. The toxic water had been rendered nontoxic simply by treatment with alga filaments. A clearer example of the effects of plants in removing or counteracting the toxic substances of a solution would be difficult to imagine. Similar results were obtained by Dandeno (7) upon growing corn seedlings in toxic solutions of HCl and H_2SO_4.

Toxic water may be greatly improved for *Spirogyra* by previous treatment with another alga. A mass of *Ulothrix* (with a few *Spirogyra* filaments) was allowed to remain twenty-eight days in ordinary distilled water (III, 35), while a control of a similar dish with another sample of the same water, but without alga, stood beside the first. At the end of this period the *Ulothrix* was removed and *Spirogyra* from the stock culture was transferred to each of the two dishes. In the water which had been previously treated with *Ulothrix* the *Spirogyra* lived, albeit in rather poor condition, for twenty-two days, while in the untreated water of the control all filaments were killed within eighteen hours.

In connection with the above observations on the effectiveness of alga filaments in correcting the toxicity of ordinary distilled water, it should be noted that, in all these experiments, the amount of *Spirogyra* added, in proportion to the amount of medium employed, exerted a considerable influence upon the results. A large amount of the alga was able to live in a small amount of a decidedly toxic medium. In all such cases the filaments on the outside of the mass were killed while those within remained in good condition. Similar results have been obtained by Nägeli (20), Deherain and Demoussy (8), Bullot (6), Dandeno (7), and Bokorny (3). In the same connection, water from the stock culture of *Spirogyra*, tested by the method used in these experiments, allowed a good growth (III, 36), but seemed less favorable than the water from a vigorous culture of *Nitella* (III, 37).

Although some of the experiments which have been considered were not repeated as many times as might be desirable, the different

experiments appear to corroborate each other, and seem to throw some light on the general nature of the toxic substances present in these waters. It will be noticed that the effect of distillation and of high temperature was often different in the case of tap water and in that of ordinary distilled water. Some of these differences are summarized in TABLE IV, where a plus sign indicates *improvement*, a minus sign indicates *no improvement*. When both signs occur, different tests were in disagreement.

The facts of TABLE IV indicate that the toxic materials present in the tap water and in the ordinary distilled water here studied were, in part at least, different substances; whatever may have been the nature of these substances those in the ordinary distilled water were mainly very resistant to heat.

As to the general characteristics of the toxic substances in the ordinary distilled water, there appears no reason for doubting that they were probably, for the most part, metallic in their nature, derived in some way from the distillation apparatus. This is the conclusion reached by many authors who have studied the toxicity of ordinary distilled waters since the time of Nägeli, and such supposition introduces no difficulty in the interpretation of the experiments that are reported here; it might be expected that metallic poisons, whether dissolved as salts or ions or existing merely in suspension, would not be very sensitive to correction by heat. The effect of redistillation might be to transfer a portion of the poisons from retort to receiver, thus leaving the distillate more or less toxic, and at the same time concentrating another portion (nonvolatile with steam and not sensitive to the temperatures employed) in the retort.

It has been shown by Livingston *et al.* (**11**), Livingston (**12**), Wheeler and Breazeale (**37**), Schreiner and Reed (**27, 28, 29**), Shorey (**30, 31**), and Lathrop (**26**), that certain organic substances present in various soil extracts are toxic to wheat seedlings. Since, as was pointed out by Livingston (**12**), tap water may be considered as a natural soil extract, the possibility is suggested that it may be toxic on account of poisons derived from the soil. At the same time, it is of course probable that the toxicity of the Heidelberg tap water here tested may have been due, to a greater or less extent, to substances having their origin in the supply pipes.

It may be supposed that somewhat complex poisons emanating from the soil would be largely volatile (either as such or as their decomposition products, *e. g.*, ammonia), and would thus be removed from the retort in the process of distillation. If such volatile material were still toxic and were retained in the receiver, the distillate would of course exhibit toxic properties. But the logical possibilities are here very numerous and a more detailed *a priori* consideration of this problem would be out of place without much more thorough experimentation than is now available.

The toxicity of the waters here dealt with was probably due, in every case, to more than one substance; different portions of tap water varied in their injurious effects, different portions of ordinary distilled water were differently affected by redistillation in glass, and other lines of evidence might be deduced from the present studies to indicate that both waters contained more than one kind of injurious material.

It appears highly probable that the beneficial effect on the ordinary distilled water of chalk, lime, agar, sphagnum moss, soil, and finely divided carbon, was due to their adsorptive action, a conclusion similar to that reached in the work of Nägeli (**20**), Breazeale (**5**), Livingston *et al.* (**11**), and Livingston (**12**). In some cases all the poisonous substances seem to have been removed from solution, for the treated water was entirely nontoxic. The nontoxic water obtained by the distillation of tap water from animal charcoal contained more dissolved electrolytic material than did the toxic water prepared by simple distillation, as was shown by conductivity measurements kindly made upon these waters by Dr. W. Fraenkel, but the total salt content of the charcoal-treated water was calculated to be less than 0.01 per cent, so that the removal of toxicity seems not to be related to an increase in salt content. Furthermore, similar decrease in the toxicity of the water was brought about by substances which were chemically very unlike the charcoal used in these distillations. The small amount of chalk that failed to produce improvement when added to ordinary distilled water was much in excess of the amount dissolved, so that the marked beneficial effect of a larger amount of chalk cannot be considered as due to the addition of calcium to the water. The improvement produced by colloidal platinum and

by agar jelly appears also to have been due to adsorptive action, though a discussion of this matter will not be given place here. It is interesting to remark that addition of sand, cotton, and filter paper failed to produce any correction of the ordinary distilled water of these experiments, although these substances have been found by Nägeli (20), Dandeno (7), True and Oglevee (36) and Jensen (9), to be active in improving other toxic solutions. The difference thus noted may be of course due either to a difference in the toxic substances dealt with or to differences in the solids used. Breazeale (5) obtained similar negative results upon adding quartz flour, filter paper, paraffin, or sand to solutions of H_2SO_4 which were toxic to corn seedlings. He suggested that the failure of these substances to reduce the toxicity of the solutions was due to the high concentration of H_2SO_4 necessary to produce toxic action, and the consequent relatively slight adsorption of the acid by the solids. This explanation can not, however, apply to the results described above, since in these experiments the amounts of the toxic substances were extremely small.

In considering the behavior of *Spirogyra* in different nutrient media (see p. 335), it has been observed that the optimum concentration of the solution appeared to be a function of the kind of water used. With solutions that contained salts in the proportions adopted by Molisch and Crone and were prepared with non-toxic water, the optimum concentration for the growth of *Spirogrya* was found to be from 0.05 to 0.1 per cent, while similar solutions prepared with ordinary distilled water exhibited an optimum concentration ten times as great. It thus appears that the toxicity of the ordinary distilled water was more or less overcome by the larger amount of nutrient salts present and that, conversely, the retarding effect of relatively high salt concentration was counterbalanced by the presence of the toxic material of the water. The influence of dissolved salts in overcoming the toxicity of solutions has been noted by other authors,* but the whole question here raised is not at present in a state to warrant an attempt at detailed discussion. It is obviously related to the general problem of physiological antagonism. Some points on salt antagonism will be brought forward in the succeeding section.

* Breazeale (4), Livingston *et al.* (11), Livingston (12), Sumner (35), Schreiner and Reed (28), and Schreiner and Skinner (32, 33).

The beneficial effect of the action of alga filaments, in rendering originally toxic water less toxic to other filaments of the same or another form (see p. 341–2), has been noted already. While the evidence at hand suggests the probability that this effect may be due to an absorptive (or adsorptive) action on the part of the filaments,—Nägeli (**20**), Deherain and Demoussy (**8**),—and that it may be therefore directly comparable with similar effects produced by other solids with very large surface exposure, it must be nevertheless remembered that such an effect may be also partially or wholly due to chemical alterations of the originally toxic substances, as if brought about by enzymatic or other material emanating from the plant. No evidence is available for a decision in this matter. The effects produced upon *Spirogyra* in toxic water, by sediment from the stock culture of this plant (III, 33) or from a *Nitella* culture (III, 32), together with the comparative growth of the alga in water from the two cultures just mentioned (III, 36, 37), may suggest a possible excretion from *Spirogyra* of material more harmful to itself than to *Nitella*. It appears that the question of toxic and antitoxic excretions may enter into problems of algal growth just as it has come to play so important a part in the physiology of the bacteria and fungi and, recently, in that of the higher plants.

III. SALTS

It has been frequently shown for both animals and plants that the toxicity of many solutions of single salts may be counteracted by the presence of other salts in the same solution, without regard to the physiological effect of the latter salts when employed singly.

Loew (**15**, **16**, **17**, **18**) would restrict this conception of the antagonistic action of two ions to the single case of calcium and magnesium. Benecke (**1**) showed that the toxicity of other salts than those of magnesium is counteracted by calcium, but ascribed the antagonistic action to calcium only. Osterhout (**21**, **22**, **23**, **24**, **25**) has, however, observed antagonism between fourteen different pairs of ions, including potassium, sodium, ammonium, calcium, magnesium, strontium, and barium. Loeb (**13**) reported that the toxic effect of a pure NaCl solution, on the eggs of *Fundulus*, was partly counteracted by each of a number of salts, including

the bases: calcium, barium, magnesium, strontium, cobalt, aluminium, lead, zinc, and chromium.

The experimentation about to be reported, upon the relation to *Spirogyra* of the salt content of the medium, is merely qualitative, the aim being to gain some knowledge of a number of possible conditions in algal cultures rather than to investigate any of the chemical questions here encountered. No attention has been given to the chemical characteristics of the salts employed, the solutions having been prepared on the percentage basis, and the terminology of percentage will be retained in the discussions. The experiments are partly repetitions of those of Benecke (1). Aside from the effect of dissolved salts to counteract the toxicity of salt solutions, these experiments also include a number of tests bearing upon the influence of undissolved materials upon this same sort of toxicity.

Throughout these experiments no water was used which had not been shown to be nontoxic; it had all been distilled from animal charcoal in glass, condensed in glass, and collected in glass. Precautions were always taken to avoid possible contamination of the dishes used, especially in the case of the cultures without calcium. All salts used, except the $CaCO_3$, were those of Merck. The experiments are listed, with their results, in TABLE V.

For the study of the influence of calcium in the medium a calcium-free solution was prepared from equal portions by weight of KNO_3, K_2HPO_4, KCl, and $MgSO_4$. This solution was tested in two concentrations, 0.4 and 0.1 per cent (of total salt content by weight), and both proved highly injurious to the *Spirogyra* here employed (V, 1). The addition of calcium-free animal charcoal produced no improvement in either case (V, 2), but common wood charcoal, pulverized, which was shown by test to contain some calcium, improved the weaker solution (V, 3). In the weaker solution a small amount of $CaCl_2$ produced about as marked improvement as did wood charcoal (V, 4), a trace of $CaCO_3$ allowed still better growth (V, 5), while an abundance of the last-named, only slightly soluble salt kept the alga in excellent condition during the entire period of the experiment—35 days (V, 5). These same experiments, performed with solutions prepared from ordinary distilled water, gave the same results, except that the

solutions thus made were all more toxic than those from nontoxic water.

A solution similar to the one without calcium, but with magnesium also absent, was prepared with equal parts by weight of KNO_3, K_2HPO_4, and KCl. This was more toxic than the calcium-free solution with magnesium present; both 0.4 and 0.1 per cent concentrations were fatal to the plant within two days (V, 6). Animal charcoal produced no improvement in the stronger solution (V, 7). The addition of calcium carbonate in considerable excess of its solubility enabled the plant to grow for several days (V, 8). A solution containing 0.1 per cent of the three potassium salts and 0.1 per cent of $CaCl_2$ was fatal within three days (V, 9), while the addition of 0.1 per cent of $MgSO_4$ to the 0.3 per cent solution of potassium salts prolonged the life of the organism for six days, but did not completely counteract the toxicity (V, 10). The last is a repetition of the first experiment of this section, where the organism lived 28 days (V, 1).

A simple solution of magnesium sulphate was fatal to the plant in concentrations ranging from 0.4 to 0.01 per cent (V, 11). Animal charcoal produced improvement only in the weakest of these concentrations (V, 12). Wood charcoal (with some calcium present) slightly improved both the 0.1 and the 0.4 per cent solutions (V, 13). While the simple solution of $MgSO_4$ killed the organism in two or three days, a 0.4 or 0.1 per cent solution of $MgSO_4$ plus 0.1 per cent of $CaCl_2$ (V, 14), as well as 0.1 per cent solution of $MgSO_4$ plus 0.1 per cent of KCl (V, 15), allowed the alga to live fourteen days. In these experiments the addition of potassium was just as efficient in counteracting the toxicity of magnesium as was the addition of calcium.

To determine whether the effect of $CaCO_3$ in counteracting the toxicity of $MgSO_4$ is to be considered as due solely to the addition of calcium to the solution or partially to other causes, the following experiments were carried out. A saturated solution of $CaCO_3$ was prepared and to this was added 0.4 per cent of $MgSO_4$. At the same time, to a 0.4 per cent solution of $MgSO_4$ was added an excess of finely divided $CaCO_3$. In the former solution, the alga was markedly injured within nineteen hours, and was totally dead at the end of fourteen days (V, 16). In the solution contain-

ing solid $CaCO_3$, however, the alga remained in good condition for seven days and had many filaments apparently uninjured at the end of fifteen days, when the experiment was discontinued (V, 17). These and other lines of evidence obtained during the progress of this work seem to indicate that the beneficial effect of the powdered $CaCO_3$ was due in part to other causes than the mere addition of calcium to the solution, probably to its adsorptive action. This suggestion is further supported by the results of Livingston *et al.* (11) Snyder and Cook (34), Breazeale (5), and Schreiner and Reed (28).

The colloidal solution of platinum already referred to rendered a 0.01 per cent solution of $MgSO_4$ decidedly less toxic (V, 18), but the addition of 0.008 per cent $PtCl_4$ to a 0.01 per cent $MgSO_4$ solution produced no observable improvement.

Although, as was noted above, the addition of 0.1 per cent of KCl rendered a 0.1 per cent solution of $MgSO_4$ less toxic, a 0.1 per cent solution of KCl alone was decidedly more toxic than this mixture (V, 20), and a 0.4 per cent solution of KCl was fatal within two days (V, 19). This result is in agreement with the work of Osterhout (22). The colloidal solution of platinum produced improvement in a 0.1 per cent solution of KCl (V, 21). Addition of 0.1 per cent of $CaCl_2$ to a 0.1 per cent solution of KCl rendered the latter slightly less toxic (V, 22), although a 0.2 per cent solution of $CaCl_2$ was fatal within a few hours or days (V, 23).

Two experiments were made bearing upon the relative endurance, in solutions of the above salts, of samples of *Spirogyra* originally from the same source, but subjected for a time to different surroundings. One portion of an alga culture which had been growing vigorously for sixty-six days in 0.1 per cent Crone's solution was rinsed in nontoxic water and placed in a solution containing 0.1 per cent of KCl and 0.1 per cent of $CaCl_2$ (V, 24) and another portion of the same culture was likewise rinsed and placed in a 0.2 per cent solution of $CaCl_2$ (V, 25). On the following day were added to both of these cultures samples of *Spirogyra* from the stock jar. In both cases the solution was injurious to both samples of the alga, but decidedly less so to the filaments from Crone's solution than to those from the stock culture (V, 22, 23). This result again emphasizes the well-known fact, already men-

tioned, that the internal conditions or the physiological state of organisms used in experimentation of this sort must always be taken into account.

In this portion of the work, no evidence was obtained for a specific action of the calcium salts in antagonizing other salts. The action of $CaCl_2$ in antagonizing $MgSO_4$ and KCl was no more pronounced than was that of KCl in counteracting the toxicity of $MgSO_4$ and of $CaCl_2$, and that of $MgSO_4$ in counteracting the toxicity of KCl and of $CaCl_2$. These results seem strongly to support the conception of Loeb (**14**) and Osterhout (**22, 25**) of the importance of physiologically balanced solutions. $MgSO_4$, $CaCl_2$, and KCl, when used separately, and a mixture of three potassium salts were all extremely toxic in the present investigation, but when the salts of any two of these bases were brought together (as in combinations of $MgSO_4$ and $CaCl_2$, $MgSO_4$ and KCL, KCl and $CaCl_2$) the resulting solution was much less toxic. A mixture of the three potassium salts here employed was as toxic as KCl alone and only when salts of all three metals were present in favorable proportions was good growth obtained. Results similar to these were reported by Bokorny (**2**). As it has been shown by several workers that *Spirogyra* requires calcium, these facts cannot be interpreted by supposing that this alga differs from higher plants in being able to survive in the absence of this element.

The improvement produced in the weaker toxic salt solutions by animal charcoal may be considered as probably due to the adsorptive action of the finely divided carbon. The beneficial effect of the addition of colloidal platinum to toxic solutions may have been likewise due to its adsorption of the toxic salts.

The author is gratefully indebted to Prof. B. E. Livingston for helpful suggestions in the preparation of this paper.

SUMMARY

1. Cultures of *Spirogyra longata* (Vauch.) Kg. were kept under laboratory conditions in a vigorous state during the entire period of the experiments,—more than 60 days. Crone's solution was the best of the nutrient solutions tested, and that of Molisch was almost as satisfactory, but the solution of Sachs seemed slightly

less favorable, while that of Knop was distinctly unfavorable, probably because of its marked acidity.

2. Both the tap water and the ordinary distilled water of the Heidelberg Institut were markedly toxic to this *Spirogyra*, even after distillation (or redistillation) from glass to glass.

3. The toxicity of the tap water was partially removed by concentrating the water to a fraction of its original volume, and was entirely removed by heating to 144° C. or by distillation in glass from animal charcoal; but the water was not greatly improved by simple distillation or by heating to 100° C. The toxicity of ordinary distilled water was partly or wholly corrected by the presence in the culture of chalk, lime, solid agar, dry sphagnum moss, colloidal platinum, or of other adsorbents; it was partially corrected by redistillation or by heating to 144° C. The presence in the cultures of filter paper, cotton, sand, kaolin, or $CaCl_2$ was without effect, and redistillation in glass from animal charcoal did not produce nontoxic water.

4. The results obtained seem to indicate that the toxic materials present in the tap water were mostly volatile (perhaps organic substances derived from the soil), with a relatively small amount of nonvolatile material probably emanating from the supply pipes. The toxic substances in the ordinary distilled water were mostly nonvolatile (probably derived from the supply pipes and from the still), with a small amount of volatile matter carried from retort to receiver with the distillate.

5. When nontoxic water was used, the best growth was obtained in nutrient solutions containing from 0.05 to 0.1 per cent of total salts; but when ordinary distilled water was used, the optimum concentration of similar nutrient solutions was ten times as great.

6. With KNO_3, K_2HPO_4, KCl, $MgSO_4$, and $CaCl_2$ (singly or in various combinations), a mixture of the three potassium salts was as toxic as KCl alone; KCl, $MgSO_4$, and $CaCl_2$ were all extremely toxic when used singly; but mixtures of any two of these three were less toxic than the solution of a single one. Good growth was obtained only when salts of all three metals were present in favorable proportions. The toxicity of $MgSO_4$ appeared to be as completely counteracted by KCl as by $CaCl_2$.

7. Weak solutions of certain toxic salts were improved by the addition of calcium-free animal charcoal or colloidal platinum. It seems indicated that the effect of powdered $CaCO_3$, in counteracting toxicity due to nutrient salts, was due in part to the adsorptive action of the solid.

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TABLE I

CULTURES OF SPIROGYRA IN NUTRIENT MEDIA PREPARED WITH DISTILLED WATER

No. of culture	Nature of culture medium			Duration of cultures, *days*	Condition of plants *
	Author and concentration, *per cent of total salts in medium*	Kind of distilled water used in medium	Agar added to medium, *per cent by weight*		
1	Sachs, 0.05	ordinary	—	3	*D*
2	0.05	nontoxic	—	48	*E*
3	0.10	ordinary	—	10, 20	*Pd, Pd*
4	0.10	do.	1.0	34	*P*
5	0.10	nontoxic	—	40, 18, 44	*E, Eg, Gp*
6	0.50	ordinary	—	3,14	*D, D*
7	0.50	do.	1.0	60	*E*
8	0.50	nontoxic	—	50	*G*
9	1.00	ordinary	—	2	*D*
10	1.00	nontoxic	—	44	*P*
11	Knop, 0.05	ordinary	—	3, 3	*D, D*
12	0.05	nontoxic	—	48	*Gp*
13	0.10	ordinary	—	3	*D*
14	0.10	do.	1.0	49	*Pd*
15	0.10	nontoxic	—	18, 31, 44, 11	*G,† Pd, Pd, Pd*
16	0.50	ordinary	—	18, 49, 1	*G,† P, D*
17	0.50	nontoxic	—	23, 2	*Pd, D*
18	1.00	ordinary	—	9	*Pd*
19	1.00	nontoxic	—	1	*D*
20	Molisch, 0.05	ordinary	—	3	*D*
21	0.05	nontoxic	—	48	*E*
22	0.10	ordinary	—	7	*Pd*
23	0.10	do.	1.0	49	*P*
24	0.10	nontoxic	—	62, 62, 40, 40	*E, G, G, G*
25	0.50	ordinary	—	55, 48, 22, 14	*G, P, P, Pd*
26	0.50	do.	1.0	66	*E*
27	0.50	nontoxic	—	50	*Eg*
28	1.00	ordinary	—	46	*P*
29	1.00	nontoxic	—	51	*P*
30	Crone, 0.05	ordinary	—	3	*D*
31	0.05	nontoxic	—	48	*E*
32	0.10	ordinary	—	3	*D*
33	0.10	do.	1.0	18	*Pd*
34	0.10	nontoxic	—	23, 40, 40, 51	*E, E, E, G*
35	0.50	ordinary	—	48, 13, 14	*Eg,† Pd, Pd*
36	0.50	do.	1.0	60	*E*
37	0.50	nontoxic	—	50	*P*
38	1.00	ordinary	—	82	*G*
39	1.00	nontoxic	—	51	*Gp*

* The condition of the plants is indicated as follows: *E*, excellent, *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters—as, *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters; thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† The amount of medium used was unusually small, in comparison with the number of filaments in the culture.

TABLE II

CULTURES OF SPIROGYRA IN TAP WATER, WITH TREATMENTS AND ADDITIONS

No. of culture	Treatments of, and additions to, tap water	Duration of cultures, *days*	Condition of plants*
1	Untreated	54, 4, 1	*P, D, D*
2	Distillate from glass to glass	12, 24, 2	*P, Pd, D*
3	As 2, first portion	3, 2	*P, D*
3*a*	As 2, middle portion	3, 12	*Gp, Gp*
3*b*	As 2, final portion	12, 3	*P, D*
4	Boiled to 1/10 orig. vol.	15, 12	*Eg, P*
5	Evaporated to 1/7 orig. vol.	1	*G*
5*a*	Untreated (control to 5)	1	*P*
6	Evaporated to 1/6 orig. vol.	1	*Gp*
6*a*	Untreated (control to 6)	1	*Pd*
7	Evaporated to 1/3 orig. vol.	1	*D*
7*a*	Untreated (control to 7)	1	*D*
8	15 mins. at 144° C.	37	*E*
8*a*	Untreated (control to 8)	37	*Pd*
9	45 mins. at 100° C.	46	*Pd*
10	Distillate from animal charcoal, glass to glass	1 to 44	*E, E, E, E, Eg, Eg, Eg, Eg, G, P*
11	Boiled with animal charcoal to 1/8 orig. vol., filtered	26	*Eg*
11*a*	Distillate from 11	26	*E*
11*b*	Do., charcoal from 11 added to culture.	26	*Gp*
12	As 11, filtered	16	*G*
12*a*	As 11, not filtered	16	*Gp*
12*b*	As 11*a*	16	*G*
12*c*	As 11*b*	16	*Gp*
13	Untreated plants from stock jar†	6	*G*
14	As 13, but plants from Crone's sol.†	6	*E*

* The condition of the plants is indicated as follows: *E*, excellent; *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters, as: *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters; thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† Nos. 13 and 14 were simultaneous, in the same dish.

TABLE III

CULTURES OF SPIROGYRA IN ORDINARY DISTILLED WATER, WITH TREATMENTS AND ADDITIONS

No. of culture	Treatments	Additions	Duration of cultures, *days*	Condition of plants*
1	Untreated	—	54, 9, 6, 1	*Gp, D, D, D*
2	Distillate, glass to glass	—	8	*G*
2*a*	Boiled, concentrated, residue from 2	—	1	*D*
3	Distillate, glass to glass, to 1/8 orig. vol., first portion	—	1	*D*
3*a*	As 3, middle portion	—	4	*Eg*
3*b*	As 3, final portion	—	4	*G*
3*c*	Boiled, contentrated to 1/8 orig. vol., residue from 3	—	1	*D*
4	Distillate, glass to glass, to 1/9 orig. vol., first portion	—	9	*G*
4*a*	As 4, middle portion	—	9	*P*
4*b*	As 4, final portion	—	9	*P*
4*c*	As 4, all portions mixed	—	9	*P*
4*d*	Distillate, glass to glass	Salts to form 0.5 *p.c.* Crone's sol.	9	*D*
4*e*	Boiled, concentrated, residue from 4*b*	—	9	*D*
4*f*	As 4*e*	Salts to form 0.5 *p.c.* Crone's sol.	1	*D*
5	Distillate, glass to glass, to 1/16 orig. vol., 1st portion	—	12	*Eg*
5*a*	As 5, middle portion	—	12	*Pd*
5*b*	As 5, final portion	—	12	*D*
5*c*	As 5, all portions mixed	—	12	*P*
5*d*	Distillate, glass to glass	Salts to form 0.5 *p.c.* Crone's sol.	12	*Gp*
5*e*	Boiled, concentrated, residue from 5*b*	—	12	*P*
5*f*	As 5*e*	Salts to form 0.5 *p.c.* Crone's sol.	1	*D*
6	Distillate, glass to glass, first portion	—	4	*Pd*
7	Distillate from animal charcoal, glass to glass	—	46	*E*
8	As 7, to 1/8 orig. vol., first portion	—	2, 12	*D, D*
8*a*	As 8, middle portion	—	3, 12	*G, Pd*
8*b*	As 8, final portion	—	12, 1	*P, D*
8*c*	Boiled with animal charcoal, filtered, concentrated residue from 8*b*	—	3, 12	*P, P*
9	Boiled 3 mins.	—	1	*D*
10	Boiled with animal charcoal, not filtered	—	1	*D*
11	15 mins. at 144° C.	—	39	*G*
12	Untreated	Nontoxic water to double vol.	2, 1, 1	*D, D, D*
13	*do.*	*Nitella* water to double vol.	1	*D*

TABLE III, *continued.*

No. of culture	Treatments	Additions	Duration of cultures, *days*	Condition of plants*
14	*do.*	Colloidal Pt sol. to double vol.	26	*Gp*
15	*do.*	Filter paper	10, 1	*Pd, D*
16	*do.*	Cotton	1, 2	*D, D*
17	*do.*	Kaolin	1, 1	*D, D*
18	*do.*	Quartz sand	1, 1	*D, D*
19	*do.*	Chalk, trace	2	*D*
20	*do.*	*do.*, excess	5	*D*
21	*do.*	*do.*, abundance	23, 56	*E, Eg*
22	Shaken with chalk, decanted after 3 hours	—	4	*D*
22*a*	As 22, decanted after 10 days	—	9, 10	*G, Pd*
23	Shaken with wood charcoal, decanted	—	53	*P*
23*a*	As 23, decanted after 4 days	—	39	*G*
24	Shaken with soil, decanted after several days	—	44	*Gp*
25	Untreated	Lime, abundance	2	*E*
26	*do.*	$CaCl_2$, O 0.4 *p.c.*	3	*D*
27	*do.*	Agar sticks	20	*E*
28	*do.*	Agar, not hardening	1	*D*
29	*do.*	Agar, 1 *p.c.*, alga on surf.	54	*G*
30	*do.*	Agar, 2 *p.c.*, as 29	3	*D*
31	*do.*	Sphagnum moss	23, 23, 9	*E, E, Pd*
32	*do.*	*Nitella* sediment	48	*Gp*
33	*do.*	*Spirogyra* sediment	5	*D*
34	*do.*		1	*D*
34*a*	Water from 34, alga removed	Alga replaced by fresh material from stock jar	1	*D*
34*b*	Water from 34*a*, alga removed	*do.*	2	*D*
34*c*	Water from 34*b*, alga removed	*do.*	15	*P*
34*d*	Water from 34*c*, alga removed	*do.*	44	*E*
35	Treated with *Ulothrix*,† removed after 28 days	*do.*	22	*P*
35*a*	Untreated (control to 35)	—	1	*D*
36	Water from stock *Spirogyra* culture	—	52, 47, 9, 4	*E, Eg, G, Gp*
37	Water from *Nitella* culture	—	52, 9	*E, E*

* The condition of the plants is indicated as follows: *E*, excellent; *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters—as, *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters; thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† *Ulothrix* killed except in center of mass.

TABLE IV

EFFECT OF VARIOUS TREATMENTS OF TAP AND DISTILLED WATER UPON THEIR TOXICITY

Treatment	Tap Water	Ordinary Distilled Water
Distilled in glass (distillate)	−	−+
Distilled in glass (undistilled residue)	+	−
Distilled with carbon (distillate)	+	−+
Distilled with carbon (undistilled residue)	+	−
Heated to 144° C.	+	+*

* Still toxic, but much improved.

TABLE V

CULTURES OF SPIROGYRA IN SALT SOLUTIONS PREPARED FROM NONTOXIC WATER, WITH AND WITHOUT ADDITIONS

No. of culture	Salts in solution, per cent				Additions	Duration of culture, days	Condition of plants*
	KNO_3	K_2HPO_4	$MgSO_4$	KCl			
1†	0.1	0.1	0.1	0.1	—	13, 28, 8	*Gp, Gp, Pd*
1*a*	0.025	0.025	0.025	0.025	—	26, 29	*Gp, P*
2	0.1	0.1	0.1	0.1	Animal charcoal‡	11	*Pd*
2*a*	0.025	0.025	0.025	0.025	*do.*	26	*Gp*
3	0.1	0.1	0.1	0.1	Wood charcoal¶	15	*Pd*
3*a*	0.025	0.025	0.025	0.025	*do.*	35	*G*
4	*do.*	*do.*	*do.*	*do.*	$CaCl_2$	28	*G*
5	*do.*	*do.*	*do.*	*do.*	$CaCO_3$, trace	35	*Eg*
5*a*	*do.*	*do.*	*do.*	*do.*	$CaCO_3$ abundance	35	*E*
6	0.133	0.133	—	0.133	—	2	*D*
6*a*	0.033	0.033	—	0.033	—	2	*D*
7	0.133	0.133	—	0.133	Animal charcoal‡	3	*D*
8	*do.*	*do.*	—	*do.*	$CaCO_3$	6	*P*
9	0.1	0.1	—	0.1	$CaCl_2$ 0.1 *p.c.*	3	*D*
10	*do.*	*do.*	0.1	*do.*	—	6	*Pd*
11	—	—	0.4	—	—	3, 2	*D, D*
11*a*	—	—	0.1	—	—	3, 2	*D, D*
11*b*	—	—	0.05	—	—	2	*D*
11*c*	—	—	0.01	—	—	6, 2	*Pd, D*
12	—	—	0.4	—	Animal charcoal‡	2	*D*
12*a*	—	—	0.1	—	*do.*	2	*D*
12*b*	—	—	0.05	—	*do.*	2	*D*
12*c*	—	—	0.01	—	*do.*	9, 5	*P, P*
13	—	—	0.4	—	Wood charcoal¶	15	*P*
13*a*	—	—	0.1	—	*do.*	6	*Pd*
14	—	—	0.4	—	$CaCl_2$ 0.1 *p.c.*	15	*P*
14*a*	—	—	0.1	—	*do.*	14	*Pd*
15	—	—	*do.*	0.1‖	—	14	*P*
16	—	—	0.4	—	$CaCO_3$ to saturation	14	*Pd*
17	—	—	*do.*	—	$CaCO_3$, in excess	15	*Gp*
18	—	—	0.01	—	Colloid. sol. Pt 90 *p.c.*	19	*Gp*
18*a*	—	—	*do.*	—	Pt Cl_4 0.008 *p.c.*	2	*D*
19	—	—	—	0.4	—	2	*D*
20	—	—	—	0.1	—	6	*Pd*
21	—	—	—	*do.*	Colloid. sol. Pt	12	*P*
22	—	—	—	*do.*	$CaCl_2$ 0.1 *p.c.*	7, 6	*Gp,*° *Pd*
23	—	—	—	—	$CaCl_2$ 0.2 *p.c.*	7, 1	*D,*△ *D*
24	—	—	—	0.1	$CaCl_2$ 0.1 *p.c.*	7	*G*°
25	—	—	—	—	$CaCl_2$ 0.2 *p.c.*	7	*G*△

* The condition of the plants is indicated as follows: *E*, excellent; *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters—as, *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters, thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† Culture 23 days old showed no calcium with acetic acid and ammonium oxalate.

‡ A culture 14 days old showed no calcium.

¶ Containing some calcium.

‖ Showed no calcium.

° *Spirogyra* from culture in Crone's solution, 66 days old, was used in 24 and 25. The first test of the stock material (22) and of material from Crone's solution (24) were simultaneously carried out in the same dish.

△ The second test of the stock *Spirogyra* (23) and of material from Crone's solution (25) were simultaneously carried out in the same dish.

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The production of a promycelium by the aecidiospores of Caeoma nitens Burrill

Otto Kunkel

Introduction

Except in the genus *Endophyllum*, so far as known, the aecidiospores of the rusts give rise to sporophytic mycelium. Its sporophytic nature is shown by the fact that each cell contains two nuclei that have been produced by successive conjugate divisions of the two nuclei in the aecidiospore. The aecidiospores of some of the species of *Endophyllum*, however, produce a promycelium. Its cells are uninucleate and belong, therefore, to the gametophytic generation. This fact has been well established through the work of Tulasne (**10**), Sappin-Trouffy (**8**), Maire (**5**) and others. Hoffmann has shown that nuclear fusion occurs in the aecidiospores and that this fusion nucleus divides to form the nuclei of the sporidia. This interesting form has received considerable attention in recent years, because its life history offers suggestions regarding evolution in the rusts.

While engaged in a study of the effects of media on the germination of various spores I was surprised to find that the aecidiospores of *Caeoma nitens* taken from leaves of *Rubus frondosus* Bigel. on germination produce a promycelium in much the same way as do the aecidiospores of *Endophyllum Sempervivi* Lév. My first suspicion that this might be due to the influence of the medium upon which the spores had been placed proved groundless, for further tests showed that they germinate in exactly the same way when placed in distilled water or in tap water. The production of a promycelium by these aecidiospores seems to have escaped the observation of the numerous students of this common and widely distributed rust.

Galloway (**6**) figured them germinating in water and shows that the germ tube becomes more or less septate. He did not, however, observe the production of sporidia

and his drawings hardly suggest the promycelial nature of the germ tube. Clinton (1) has also studied the germination of these spores and gives more than twenty figures, showing them in various stages of development, but he failed to find any trace of sporidia. He mentions having observed the spores in water cultures during two or three days and gives a detailed account of their germination. He also observed the germination of the spores on the moist surface of young blackberry leaves.

Olive (7) and Kurssanow (4) have investigated *Caeoma nitens* from the cytological standpoint. They have shown that sexual fusions occur in the base of the caeoma and that the aecidiospores typically contain two nuclei.

OBSERVATIONS

The aecidiospores used in this study of their germination were obtained from well infected leaves of *Rubus frondosus*. The leaves were collected in three different parts of Van Cortlandt Park, New York City. All of the spores germinated in the same fashion.

The cultures were made in Petri dishes containing distilled water, tap water, and agar media of various kinds. The spores were simply dusted over the surface of the medium and generally gave a high per cent of germination. The cultures were kept at room temperature (about 23° C.).

The promycelium ordinarily consists of five cells (FIG. 1, *a*), four of which bear sporidia. Stained preparations show that the stalk cell is without a nucleus and that the other four cells contain one nucleus each (FIG. 1, *b*), thus demonstrating the promycelial nature of the germ tube. Frequently the promycelium consists of only four cells and in that case each cell is capable of producing a sporidium (FIG. 1, *c*). There are also instances where the promycelium consists of less than four cells, but in such cases two or more sporidia are generally produced on the same cell. The promycelium sometimes consists of more than five cells (FIG. 1, *d*). Five or six sporidia are sometimes produced by the same promycelium, but this is a very rare case. In FIG. 1, *e* is shown a basidium with sporidia in different stages of development; it also shows one sporidium that has fallen off from its sterigma and has begun to germinate.

The sterigma arises as a pointed outgrowth from a promycelial cell. It tapers evenly and is generally slightly curved rather than straight. Its diameter at the base varies between three and five microns, while at the tip its diameter measures from one and one half to two and one half microns. It normally reaches a length of from fifteen to twenty microns, but in some cases it becomes much longer.

The sporidium, as is the rule in the rusts, is a typically kidney-shaped spore, slightly narrower at its basal than at its distal end. Maturing in a short time after the aecidiospore begins to germinate, it soon falls off from its sterigma and, on a moist surface, germinates

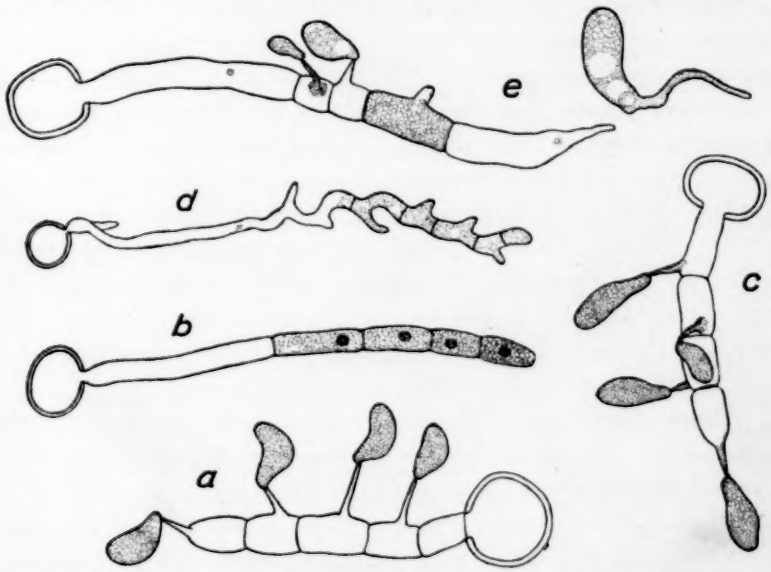

FIG. 1. *a*, Normal germinating aecidiospore, with promycelium bearing sporidia; *b*, normal uninucleated promycelium; *c*, aecidiospore giving rise to a four-celled promycelium; *d*, aecidiospore producing an abnormal promycelium; *e*, germinating aecidiospore with promycelium bearing sporidia in different stages of development.

in a few hours. Sometimes it produces a secondary sporidium, but more often it may develop a corkscrew-shaped germ tube that frequently reaches a length eight or ten times that of the sporidium. Occasionally this germ tube shows branching. The diameter of this germ tube is always much less than the diameter of the normal promycelium.

Although there is considerable variation in the length and shape of the promycelium when grown on various media, under fairly similar conditions it is rather constant both in size and in shape. Generally it reaches a length of three or four times the diameter of the spore, but sometimes it becomes much longer than this. The average diameter of the spore, based on a measurement of twenty-five spores taken at random, is twenty-one microns. The average length of the mature promycelium, based on the same number of measurements, is seventy-eight microns.

DISCUSSION

Tranzschel (9) and Clinton (2) both claim to have established by infection experiments that *Puccinia Peckiana* Howe is the teleutostage of *Caeoma nitens*. Tranzschel removed three healthy blackberry plants from the park of the St. Petersburg Forestry Institute. One of these plants he grew under a bell jar in the laboratory of the Institute; the other two plants were placed in a garden. The three plants were inoculated with spores of *Caeoma nitens*. Later in the season all of the three plants became infected with *Puccinia Peckiana*. Tranzschel had no control plants in his experiment and he makes no mention of having observed the plants in the park of the St. Petersburg Forestry Institute at the time his plants showed infection with the *Puccinia*.

Clinton transplanted blackberry plants from the forest to his greenhouse. He inoculated two of these with the aecidiospores of *Caeoma nitens* taken from infected blackberry plants. In a little less than two months both of these plants showed infection with *Puccinia Peckiana*, while several other blackberry plants that had been used as checks showed no infection. It is worth noting that three raspberry plants which he inoculated with spores of *Caeoma nitens* taken from infected raspberry plants, failed to become infected. All plants used had been free from both the *Caeoma* and the *Puccinia* during the previous summer.

It is hardly to be expected that the change from sporophyte to gametophyte should occur in two different places in the life history of the same rust, but further experiments are needed in order to settle this point.

The production of a promycelium by the aecidiospores of

Caeoma nitens suggests that it is a short-cycled rust in no way connected with *Puccinia Peckiana* and in its life history quite comparable to *Endophyllum Sempervivi*. The perennial mycelium lives over winter in the tissues of the host. Cell fusions at the base of the chains of aecidiospores produce sporophytic cells from which the aecidiospores arise by conjugate division of the two cells that fuse. This gives binucleated aecidiospores and intercalary cells. The fact that the aecidiospores germinate in a promycelium suggests that here, as in *Endophyllum*, nuclear fusion takes place in the aecidiospore and we may expect that the sporidia produced by the promycelium reinfect the host and produce a mycelium with uninucleated cells, thus completing the life cycle.

If, on the other hand, *Puccinia Peckiana* is a stage in the life history of this fungus, the problem is not so simple and no similar case is known among the rusts.

As was pointed out in the introduction, the fact that sexual fusions occur previous to the production of aecidiospores is well established. The above described germination of these spores makes clear another stage in the life history of this fungus, and raises a number of interesting questions regarding its position among the rusts. I shall test during the coming summer the evidence regarding the connection supposed to exist between *Caeoma nitens* and *Puccinia Peckiana*, and shall also undertake a cytological study of both of these forms. The wide distribution and economic importance of *Caeoma nitens*, its abundant production of spores and the ease with which they may be germinated and studied makes it a favorable object for class use. It is, therefore, highly important that all stages in its life history should be thoroughly understood.

SUMMARY

1. The aecidiospores of *Caeoma nitens* on germination regularly produce a promycelium.

2. This promycelium normally consists of five cells. The stalk cell contains no nucleus, but the other four cells contain one nucleus each.

3. Each uninucleated cell bears a sporidium on a sterigma.

4. These sporidia germinate immediately by producing either a secondary sporidium or a germ tube.

5. The production of a promycelium by these aecidiospores suggests that *Caeoma nitens* is a short-cycled rust and casts doubt on the connection supposed to exist between this fungus and *Puccinia Peckiana*.

6. *Caeoma nitens* is the only rust of the caeoma type, having aecidiospores that are known to produce a promycelium. The other rusts having aecidiospores that are known to function as teleutospores, belong to the one genus *Endophyllum*.

I am greatly indebted to Dr. R. A. Harper and Dr. W. G. Marquette for advice and criticism while engaged in this work.

COLUMBIA UNIVERSITY, NEW YORK CITY.

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A case of bud-variation in Pelargonium

A. B. Stout

(with plate 20)

The precise nature of bud-variation is not satisfactorily known. No adequate classification of the various kinds of bud-variation has been made. Recent investigations regarding the nature of plant chimeras indicate that some of the phenomena generally considered as bud-variation are associated with chimeras and are to be explained by the nature of the chimeras. Observations and experiments are now being made at the New York Botanical Garden on various types of bud-variation. In the studies on *Pelargonium* one case has arisen which seems of special interest in its bearing on the nature of bud-sports from plants that are chimeras.

Baur (1909 *a* and *b*; 1911) has recently shown that the variegation in the case of the "albomarginatae" varieties of *Pelargonium zonale* is due to the presence of white and green cells which are sharply distinct and which occupy a characteristic position in relation to each other. It has been long known that the paler tissue of these variegated plants owes its characteristics to a lack of chlorophyl in its cells. Baur shows that the plastids are present in the white cells but are colorless.

Baur further claims that this arrangement of green and white cells in the leaf can be explained by the arrangement of the corresponding tissues in the growing point and actually shows that in the plants whose leaves have layers of white cells on the exterior there is in the apex of the stem a cap of white cells over the greener cells beneath. In such plants the relative position of the white and the green cells is maintained throughout the development of the leaves.

By further study Baur (1909 *a*; 1911) found that in other cases these two kinds of cells may be variously arranged with reference to each other. In some plants various stems and leaves show a

sectoral arrangement with a more or less bilateral distribution of the two kinds of cells. In certain types of *Pelargonium* the green cells are outside as one or two layers covering the white cells. In some individuals streaks of one of the tissues are mingled with the other.

Baur is able to interpret the conditions in these various forms by using the conception developed by Winkler (1907) in his remarkable discoveries regarding the so-called chimera-nature of graft hybrids. Baur introduced the term *periclinal chimera* for the condition where the peripheral cell-layers are different from the enclosed tissues and *sectoral chimera* for the cases where there is more or less of a bilateral or radial distribution. The term *hyperchimera*, first suggested by Strasburger (1909), is used for the cases where there is a more or less intimate mixture of the different kinds of cells.

On these various chimeras of *Pelargonium*, wholly green or wholly white shoots may arise. This is due to the fact that the two kinds of cells which are maintained by the cell divisions in the meristematic regions become segregated in the growing points, the process not being essentially different from that by which peripheral, sectoral, or hyperchimeras arise.

The various types of these *Pelargonium* chimeras are familiar to horticulturists and have been propagated rather widely by cuttings, thus preserving quite uniformly the different forms. The periclinal chimeras having white peripheral cell layers are commonly cultivated forms on account of the striking effect of the white-margined leaves.

One of these varieties is known by the trade name of *Madame Salleroi*. During the summer of 1912 a plant of this type which was grown in an outdoor bed at the propagating houses of the New York Botanical Garden produced a branch in which the relative position of the two kinds of cells is reversed. When the cutting was made during the early part of the summer it possessed only leaves with the white margin. In October of that year, when first brought to the attention of the writer, the plant appeared as shown in the photograph here reproduced (see PLATE 20). Two branches, one the main and the other a lateral branch, bore leaves with the white cell layers placed externally to the green as

was the case in the plant from which the cutting was made. The leaves were quite uniform in shape and in coloration and are quite typical for the variety known as *Madame Salleroi*. Dr. L. H. Bailey kindly made the varietal determination from leaves taken from these branches and further states in a letter to the writer that this variety has not been thus far placed specifically. As shown in PLATE 20, the enclosed green cells fail to develop uniformly toward the margin of the leaves, thereby leaving an irregular marginal zone of pure white cells. In the central portion of these leaves the enclosed green tissues show through the white.

On the third branch, which is a lateral one, the leaves are quite different. They are larger and the surface is green to the extreme margin. These leaves are not, however, of a uniform green, for through the central portion of each there is an irregular palmate-shaped area of lighter green which is due to white cell-layers enclosed between the upper and the lower green layers. In other words the place relationship of the white and the green cells is here reversed from what it is in the main part of the plant. In this branch the plant has literally turned itself inside out. Microscopical examination of free-hand sections confirmed the superficial observations as to the color relations.

In the black and white plate accompanying this article the general pattern in the leaves is well shown by the different shades. Since this plant has been under observation, about twenty leaves have matured on this branch. As shown in PLATE 20 the amount and distribution of the white tissue varies in the different leaves. In some leaves there are small flecks of white scattered through the green. A few of the leaves when about half developed show traces of a dark zonal band which is a feature of various showy-leaved Pelargoniums. When these leaves are mature, however, this zonal band is faint.

Baur notes a case of bud-variation identical with this one. He states (1909 *a*, p. 333), that the plant which he designated as Pel. 9 had white-bordered leaves but produced in 1908 a branch having wholly green leaves but which were plainly of a yellowish green in the center. The anatomical studies of these leaves (1909 *a*, p. 345) showed that the white cells were enclosed by the green cells.

In the light of Baur's anatomical studies this sort of bud-variation is readily understood as due to a mechanical readjustment of the two kinds of cells already in the growing points. This particular type involves more extensive rearrangement than the cases where pure green or pure white branches are produced by the development of a bud containing only one kind of cells to the exclusion of the other. For the development of a branch reversing the position of the two kinds of cells as described above there must be a breaking out of the enclosed green cells in the growing point and a growth of both green and white cells in such a manner that the green cells surround the white cells. It may be that in this case the green cells break forth at two separate points not far distant and that in further growth they meet, enclosing the white cells.

On the main portion of the plant here shown, the mature leaves possess over their whole surfaces two peripheral cell-layers that are white. To maintain this relationship the cell divisions which give rise to these layers must occur only in planes which are at right angles to the surface of the leaf. The outer layers do not contribute to the vascular tissues and the inner green tissue does not form epidermis, a fact clearly shown by Baur. In the sporting branch, however, the green cells get to the surface and form the epidermis as well as some of the mesophyl and vascular tissue, while the white cells cease to form epidermis and now contribute only to the inner tissues. The cells preserve the green and white character of their chromatophores but take on different structures or different functions according to position and environment.

In his interesting report of results of anatomical and hereditary studies of variegated varieties of *Pelargonium*, Baur was not especially concerned with the evidence of interaction between the two kinds of cells, the white and the green, where both exist in the same leaf. His photographs, however, show the same sort of difference which have appeared so strikingly in the case here under consideration.

The marked differences between the two kinds of leaves produced on this plant (PLATE 20) make it clear that the outer layers largely determine the size of the leaves and the depth of the lobing. When the green is outside the leaf is larger, more deeply lobed and

like those which are borne on the branches that are composed purely of green tissue. When the white is outside, the leaf is much smaller and more like those leaves which are composed only of white tissue. Several plants of *Pelargonium Madame Salleroi* of the same clone as the plant producing this bud-variation have been under observation in the propagating houses. Several of these have produced leaves composed wholly of white cells. These have been of practically the same shape and size as the variegated leaves on the same plant. The pure white cells are, of course, dependent upon the green cells for carbohydrate food. In the case of a chimera relationship with the green cells enclosed, there may be mechanical and chemical stimuli to the overlying white cells that result in a slightly larger leaf. This effect is, however, not marked.

All the potentialities of a large and deeply lobed leaf are present in the green cells of a typical leaf of the *Madame Salleroi* variety. When these green cells get to the exterior those potentialities find expression, but as long as the peripheral layers of white are uniformly maintained, there is no visible evidence that these potentialities exist. Their suppression may be due chiefly to mechanical limitations imposed by the peripheral layers of white cells which decrease the number of cell divisions.

In the various plant chimeras there is an association of more or less independent and different kinds of cells. In the chimeras resulting from grafting, the two kinds of cells may be decidedly different, producing, when separate, two distinct types of leaves, but when associated together, forming leaves of still different patterns. The various chimeras produced by Winkler (1907 and 1909) and the chimera *Crataegomespilus Asnieresii* (see illustration by Baur 1911, *pl. VIII*) illustrate this phenomenon. In addition to such mechanical and physical interactions, Winkler (1910) has presented some evidence that there may be a vegetative fusion of cells in graft tissues producing what he would consider as the only true graft-hybrid, and he further holds that hybrid modifications may also result from the migration of such substances as atropin or nicotine between stock and scion.

These facts indicate that the general phenomena of plant chimeras have a very direct bearing on theories of morphogenesis

and that cellular interaction is a potent factor in influencing cell differentiation and in determining the physical characteristics of such organs as leaves.

The bud-sports which arise from the various *Pelargonium* chimeras are, we may say, rather simple cases of variation due to a mechanical rearrangement of the kinds of cells already present. Their appearance is a confirmation of the rule that like produces like in its application to cell lineage rather than evidence of spontaneous somatic mutation or variation. In this case the real variation occurs when the white cells appear as the progeny of green cells. The frequency of this spontaneous variation in *Pelargonium* (and in other cases as well) and the real nature and the causes of the process are problems for future solution.

NEW YORK BOTANICAL GARDEN.

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INDEX TO AMERICAN BOTANICAL LITERATURE

(1911–1913)

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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